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Effects of Zostera marina Roots and Leaf Detritus on the Concentration and Distribution of Pore-Water Sulfide in Marine Sediments

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Effects of *Zostera marina* roots and leaf detritus on the concentration and distribution of pore-water sulfide in marine sediments

By

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MASTER'S THESIS

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Alexandra Garcia Simpson
November 14, 2016

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A Thesis
Presented to
The Faculty of
Western Washington University

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Abstract

Sulfide toxicity, coupled with other environmental stressors, are implicated in seagrass declines worldwide. Yet, studies examining the relationship between seagrass presence and sulfide concentrations have yielded conflicting results. Interpretation of the seagrass-sulfide relationship is complicated due to the opposing effects of the root system which can increase sulfide oxidation and the burial of organic matter from the plant itself which can increase sulfide production via anaerobic sulfate reduction. To quantify the impact of eelgrass leaf detritus and the *Zostera marina* rhizosphere on pore-water sulfide concentrations, field samples of pore-water sulfide were collected in areas with and without eelgrass. To decouple the effects of live versus dead eelgrass tissue, laboratory studies were conducted over 4 weeks using 10 aquaria with or without eelgrass shoots and 0-8 pieces of *Z. marina* detritus located at 4 cm and 11 cm depth. Diffusive Gradients in Thin-Films (DGTs) were used to obtain 2D visualizations of sulfide concentrations within the sediment in relation to location of eelgrass detritus and the rhizosphere. In the field study, the presence of leaf detritus accounted for higher than average sulfide concentrations in the sediment, both within and outside eelgrass beds. In the laboratory study, the presence of live eelgrass shoots resulted in higher overall sulfide concentrations compared to aquaria without eelgrass. Sulfide concentrations, localized around the leaf detritus additions, increased with higher mass of added detritus compared to locations where no detritus was added. Sediment within the rhizosphere exhibited reduced sulfide concentrations compared sediment outside the rhizosphere. In addition, higher sulfide

concentrations were typically found at deeper depths. These results indicate why in some cases seagrasses lower sulfide concentrations and in others increase concentrations. It is likely that seagrasses are simultaneously increasing and decreasing sulfide concentrations depending on the location analyzed relative to the rhizosphere or buried eelgrass detritus.

Acknowledgements

I would first like to thank my graduate advisor, Dr. David Shull for his unwavering support and guidance throughout the Master's degree program at Western Washington University. He has been an invaluable source of knowledge guiding me through every step of this process while always having time to answer even the most miniscule of questions. His time and effort is thoroughly appreciated. Dr. Sylvia Yang provided a spark of ideas whenever thoughts were stalled on methodology, statistical analysis and writing. Her energy and support throughout this process has been incomparable and will always be admired. Dr. Brooke Love provided excellent feedback into the formation of my experiment and writing process and as such, has been an excellent boost to have on my committee.

I would also like to thank Shannon Point Marine Center for allowing me to use their facilities and in particular Gene McKeen and Horng-Yuh Lee for their assistance with experimental set-up. F. Scott Wilkinson has also been a huge help in acquiring materials while also willingly answering any question I asked. I would also like to thank Dr. Michael Rearick for providing insight into the DGT gel making process. Without his tips and tricks, I would likely still be in the DGT-making infancy stages. Laura Tripp and Mike Adamcyzk helped more with the experimental process than either could ever realize. I could not have completed this without their assistance. Melissa Ciesielski was an excellent guide to the ins and outs of the graduate program and her help will always be appreciated. Finally, I would like to thank JJ Wright for his emotional support, understanding and late-night talks about sediment chemistry.

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1.0 Introduction

Although providing numerous ecosystem services, the marine flowering plant *Zostera marina* (eelgrass) faces worldwide declines (Moore and Short 2006; Orth et al. 2006). While widespread declines have yet to occur within the Salish Sea, a few localized declines, most notably in Hood Canal and the San Juan Archipelago, have occurred (Dowty et al. 2005). Due to this decline, many of the ecosystem services provided, including coastal sediment stabilization, provision of nursery grounds for juvenile salmon (*Oncorhynchus* spp.), and habitat for economically important species such as various shellfish and Dungeness crab (*Cancer magister*), are vanishing (Short and Wyllie-Echeverria 1996; Moore and Short 2006; Orth et al. 2006). Multiple stressors such as eutrophication, limited light availability, increasing temperature, and hydrogen sulfide, can cause declines in eelgrass meadow distributions (Goodman et al. 1995; Short and Wyllie-Echeverria 1996; Koch 2001; Orth et al. 2006; Thom et al. 2008; Waycott et al. 2009; Lamers et al. 2013). Pore-water hydrogen sulfide, a known toxicant inhibiting photosynthesis in seagrasses, works synergistically with other stressors such as low light availability or hypoxia, to diminish growth, decrease biomass and cause numerous other negative physiological effects in eelgrass meadows (Bagarinao 1992; Holmer and Bondgaard 2001; Koch and Erksine 2001; Pedersen et al. 2004; Borum et al. 2005, 2014; Korhonen et al. 2012; Lamers et al. 2013).

1.1 Sulfide Production

Sulfide is produced through the anaerobic mineralization of sedimentary

organic matter. In order to respire, aerobic microbes will exploit all available oxygen within the top few millimeters of sediment (Burdige 2006; Mitsch and Gosselink 2007). Once the available oxygen in the sediment is utilized, the anaerobic microbes will capitalize on other electron acceptors for respiration, beginning with nitrate, then manganese oxide, iron oxide and sulfate (Burdige 2006; Mitsch and Gosselink 2007). It is through sulfate reduction that several species of sulfide are created, namely H_2S, HS^-, and S^{2-}.

1.2 Eelgrass Impacts on Pore-Water Sulfide

The reducing sediments that eelgrasses generally occupy allow for sulfate reduction to occur naturally (Jorgensen 1977; Terrados et al. 1999; Pedersen et al. 2004; Burdige 2006). Furthermore, the accretion of plant detritus and other organic matter can cause eelgrass beds to harbor higher levels of sulfide in comparison to other marine habitats (Harrison and Mann 1975; Pollard and Moriarty 1991; Isaksen and Finster 1996; Holmer and Nielsen 1997; Holmer et al. 2005).

Nonetheless, eelgrass employs multiple physiological defense mechanisms to prevent toxic levels of sulfide from entering its system. For instance, small "halos" of oxygen, produced through photosynthesis, diffuse out of and surround the root tips within the rhizosphere and act as barriers to the uptake of reduced compounds like sulfide (Fredricksen and Glud 2006). Unfortunately, the oxidized barriers can break down under stressful conditions and allow for sulfide intrusion into the plant (Pedersen et al. 2004).

Few studies have detailed how eelgrass influences sediment chemistry,

especially with respect to sulfate reduction and sulfide oxidation (Pagès et al. 2012). This is likely due to strong multi-dimensional spatial gradients in sulfide concentration and the complexities of the root system. We can measure how the interactions between eelgrass detritus and eelgrass rhizosphere impact the spatial dynamics of sulfide concentrations by using the relatively new technique of Diffusive Gradients in Thin-Films (DGTs). DGTs have been used in seagrass sediments (Deborde et al. 2008; Cesbron et al. 2014; Pagès et al. 2012) as an *in situ* method to quantify the distribution of various compounds including sulfide, trace metals, and nutrients within the seagrass rhizosphere.

1.3 Diffusive Gradients in Thin-Films (DGT) Technique

The DGT technique provides a two-dimensional visualization and quantification of sediment chemistry by utilizing and quantifying the diffusion of solutes into the sensor (Zhang and Davison 1995). DGTs are composed of a nitrocellulose filter membrane, a diffusive polyacrylamide hydrogel and a compound binding resin gel (Figure 1; Zhang and Davison 1995; Robertson et al. 2008). Solutes are transported by molecular diffusion through the filter membrane and diffusive gel where they then bind to the resin gel (Zhang and Davison 1995; Robertson et al. 2008). One important constraint with DGTs is that they are based on time-dependent accumulation and therefore require long deployments with precise time recording (Zhang and Davison 1995; Robertson et al. 2008). The length of time required for the binding resin gel to become fully saturated with the target solute is dependent upon the combined thickness of the diffusive gel and filter

membrane. In theory, a longer deployment time and thinner diffusive gel can lead to lower detection limits for the targeted solute (Zhang and Davison 1995; Robertson et al. 2008).

DGTs have been successfully utilized in seagrass sediments to create 2D high-resolution pore-water sulfide concentration profiles as DGTs boast a spatial resolution of better than 1 mm (Zhang and Davison 1995; Pagès et al. 2012). It would be extremely difficult to measure sulfide at that resolution using other methods such as sediment cores or pore-water sippers. Furthermore, it is nearly impossible to know the proximity of a conventional pore-water sample to eelgrass root tips where sulfide intrusion can occur because it is not possible to observe the sampler tip in relation to the location of the roots. This is important because low sulfide levels would likely be found near root tips whereas high sulfide levels could be found further away.

1.4 Objectives

This technology can provide spatial information about sulfide concentration and distribution relative to eelgrass root tips and detritus. As such, the primary objective of this study was to determine how natural processes, such as oxidation around the root zone and input of eelgrass leaf detritus, impact sulfide concentrations within eelgrass meadows. This objective led to multiple hypotheses. First, eelgrass inhabited sediments will possess higher pore-water sulfide concentrations compared to unvegetated sediments due to the accretion of organic matter. Second, decaying eelgrass leaves will locally increase pore-water sulfide

concentrations within the sediment. Third, oxidation of the sediment within the rhizosphere will locally decrease pore-water sulfide concentrations. The goal of this study is to better understand the natural processes by which eelgrass changes sediment chemistry through the provision of both organic detritus and dissolved oxygen at different locations within the sediment.

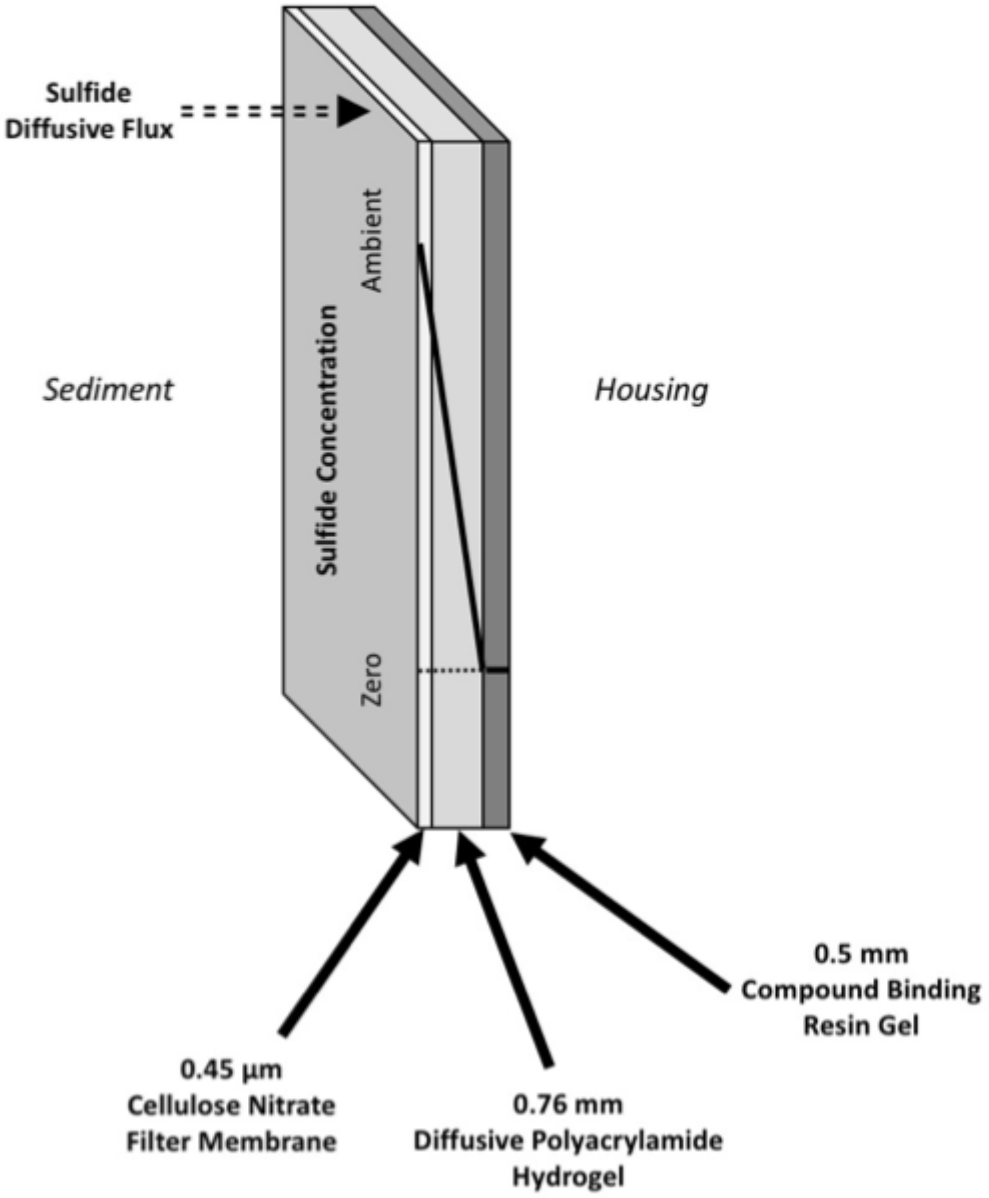

Figure 1. Schematic of the DGT gel layers. Solutes are transported from the pore-water through the nitrocellulose membrane and diffusive hydrogel before binding to the resin gel.

2.0 Methods

2.1 Site Description

With over 3,200 ha of eelgrass, Padilla Bay boasts a large percentage of the currently estimated 22,000 ha of eelgrass growing within the Salish Sea (Dowty et al. 2005; Nearshore Habitat Program 2015). Located in the northwestern portion of Washington State, Padilla Bay is included in the National Estuarine Research Reserve System due to its extensive eelgrass meadows (Padilla Bay NERR 2008). Although relatively low levels of sulfide (<1 mM) are present in Padilla Bay, previous studies on eelgrass from Padilla Bay have shown sulfide levels as low as 1 mM can impact eelgrass physiology and growth (Walser 2014).

2.2 Diffusive Gradient in Thin-Films (DGTs) Preparation

To measure pore-water sulfide concentrations, DGTs utilize three layers: a nitrocellulose filter membrane, a diffusive polyacrylamide hydrogel and an AgI binding gel (Rearick 2004; Robertson et al. 2008). Sulfide solutes are transported by diffusion through the membrane and diffusive gel before binding with the AgI resin gel to form Ag_2S. Pore-water sulfide concentrations are then calculated based on the mass of the resulting Ag_2S, the thickness of the diffusive gel, the length of time the sensors were deployed and the diffusion rate corrected for temperature.

AgI binding gels and diffusive polyacrylamide gels were constructed as detailed previously (Zhang and Davison 1995; Rearick 2004; Robertson et al. 2008). Gel molds were created using two slightly offset 17.8 cm x 10.2 cm acid washed glass separated by thin spacers. For the diffusive gels, nylon spacers with a

thickness of 0.76 mm were used and for the binding gels, stacked strips of plastic (polyethylene terephthalate) totaling 0.5 mm in thickness were used. The glass plates were then clasped together using binder clips before gel was pipetted into the gap.

For both binding and diffusive gels, a stock polyacrylamide solution composed of 35.6 g acrylamide (IBI Scientific), 1.87 g N, N-methylene bisacrylamide (G-Biosciences) and 250 mL Milli-Q water (MQ; Millipore Element) was prepared. To create the AgI binding gels, a 6.51 ml aliquot of stock polyacrylamide solution was mixed with 1.68 mL 1 M $AgNO_3$ (ACS Grade; VWR) and 38.5 µl of 10% by volume ammonium persulfate (APS; IBI Scientific). The mixture was pipetted into a prepared glass mold and placed in a drying oven at 40 °C until solidified. After cooling to room temperature, the binding gel was removed from the glass mold and immediately immersed in a ~0.2 M KI (ACS Grade; EMD Millipore) bath and kept in the dark for several hours until it became opaque. During this process, the $AgNO_3$ present initially in the gel reacts with the KI bath resulting in the formation of the AgI binding gel. After immersion, the binding gels were stored in MQ water to hydrate and rinsed 3 times during the first 24 hours after creation. The diffusive hydrogels were prepared by mixing 15.0 ml stock polyacrylamide, 15.0 ml MQ water, 350 µl 10% APS, and 12.6 µl 99% N, N, N, N-tetramethylethylenediamine (TEMED; VWR). Similarly, the diffusive mixture was pipetted into a prepared glass mold, placed in a drying oven at 40°C until solidified, cooled to room temperature and carefully removed from the glass plates. The diffusive hydrogels were

immediately placed in MQ water for storage and hydration, and rinsed 3 times in the first 24 hours after creation.

2.3 DGT Calibration

Thirty 3 cm x 3 cm DGT squares were immersed in known sulfide concentrations for 4 hours at 20 °C in a deoxygenated tank. DGTs were scanned at 300 dpi using a flatbed scanner (Epson Workforce 325) in TIFF formats and analyzed via 8-bit grayscale color intensity using ImageJ version 1.49 (Rasband 2015). Following Pagès et al. (2012), grayscale images were resized so that 1 pixel was equivalent to 1 mm x 1 mm. The theoretical sulfide uptake per square centimeter of each gel was calculated using the DGT equation (Zhang and Davison 1995):

$$C = \frac{M\,\Delta g}{DAt} \quad (1)$$

where C is the concentration in the bulk solution (mM), M is the mass of the diffused ion in the resin gel (g), Δg is the diffusive thickness (mm), D is molecular diffusion ($cm^2\ sec^{-1}$), A is the surface area of the membrane (cm^2) and t is time (seconds). A standard curve was then created relating sulfide uptake to grayscale intensity (Figure 2). The variation in the data was explained using a hyperbolic standard curve ($R^2 = 0.96$).

To determine the sulfide concentration for the DGTs used in field and laboratory studies, a modified version of the standard curve (Figure 1) was used to calculate theoretical sulfide uptake from grayscale intensities using Eq. 2:

$$U = \frac{I_g}{554.125 - 3.1789 \times I_g} \quad (2)$$

where U is the theoretical sulfide uptake concentration (mM cm^2) and I_g is the grayscale intensity. Once sulfide uptake was determined, a modified version of the DGT equation was used to calculate actual sulfide concentrations:

$$C_{DGT} = \frac{U\,\Delta g}{Dt} \quad (3)$$

where C_{DGT} is the sulfide concentration measured by the DGT, U is the theoretical uptake of sulfide concentration (mol/mL), D is the diffusion coefficient ($cm^2\ sec^{-1}$), t is time (seconds), and Δ g is the diffusive path length (cm). The diffusion coefficient was determined from Li and Gregory (1974) using HS^- diffusion values:

$$D = (0.2977\,T + 9.6823)\ 10^{-6} \quad (4)$$

where D is the diffusion coefficient ($cm^2\ sec^{-1}$) and T is the temperature of the pore water at time of deployment (°C).

2.4 DGT Probe/Aquaria Assembly

To house the gels for the field study, multiple 17.8 cm x 12.1 cm sediment probes with 15.3 cm x 9.5 cm exposure windows were created using methods found in Robertson et al. (2008; Figure 3). Similar housings were built to fit into an opening in the side of 30.5 cm x 20.4 cm x 2.6 cm aquaria resembling "ant farms" filled with sediment from Padilla Bay (Figure 3). An 18 x 12 cm opening in the side of each aquarium allowed the gel to be placed directly on the sediment and removed without disrupting the sediment fabric. DGTs for both field probes and aquaria gel holders were assembled with the AgI binding gel underlying the diffusive gel covered by a nitrocellulose membrane filter (0.45-μm pore size; Bio-Rad). The nitrocellulose membrane filter prevented larger particles from sticking to the gels while allowing solute diffusion.

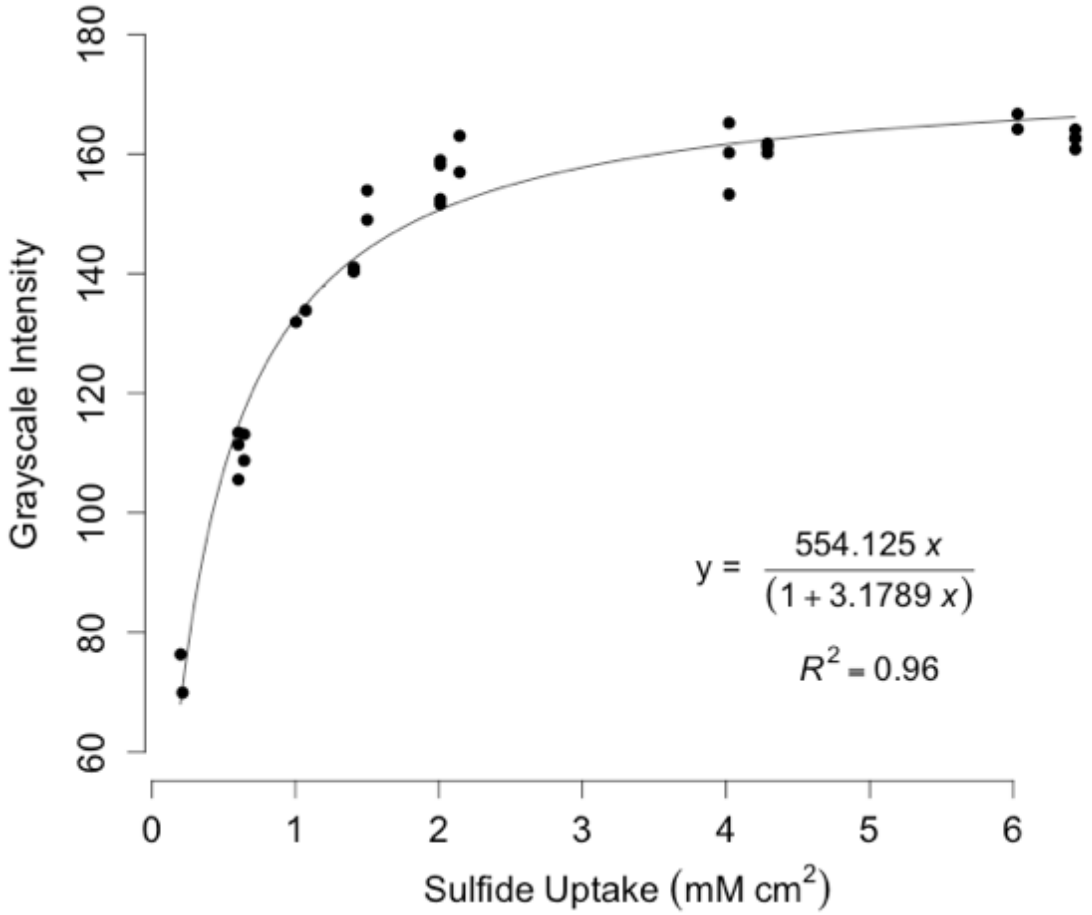

Figure 2. Diffusive gradients in thin-film (DGT) standard curve calibration. Grayscale intensity (0-255) of DGTs were related to known concentrations of sulfide (mM cm^2). The fitted line is the calibration standard used for all DGTs. The corresponding equation was used to calculate sulfide concentrations in both experimental and field-deployed DGTs.

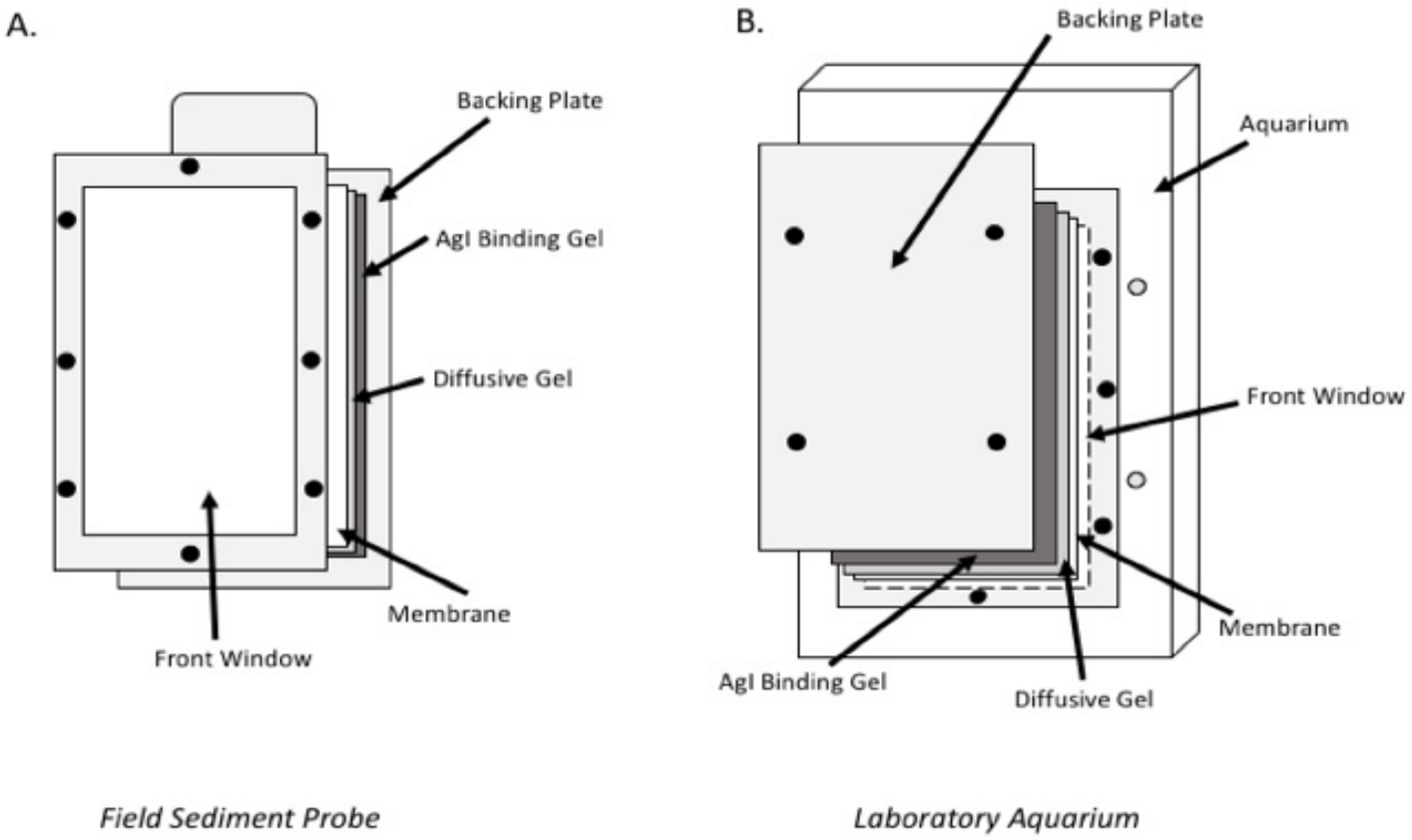

Figure 3. Diagrams of field sediment probe and laboratory aquarium. *A* refers to the DGT field probe and *B* refers to the setup of the DGT in the laboratory study

2.5 Field Study

DGT probes were deployed twice during July 2016 at various sites within Padilla Bay, WA to determine levels of naturally occurring pore-water sulfide concentrations. A total of 8 field DGT probes were deployed during low tide and collected before the flood tide covered the site, approximately four hours later. All DGT probes were deployed and collected within a 30-minute timeframe to minimize any time effects during each day of deployment. Sites were selected based on proximity to eelgrass; 3 DGTs were deployed in bare spots and 5 DGTs were deployed adjacent to eelgrass rhizomes. Bare spots were considered "bare" only if no eelgrass was growing in ca. 1-m radius from the location of the DGT probe. The probes were inserted into the sediment until the top of the DGT was a few cm above the sediment surface. Immediately after retrieval, probes were rinsed with seawater to remove excess sediment. The probes were deconstructed and the binding gels were subsequently scanned between two transparency sheets and analyzed as outlined in section 2.3.

To analyze the sediment adjacent to the field-deployed DGTs for detritus and root presence, a vertical slab of sediment adjacent to the DGT location was collected. Vertical and horizontal variations in sediment properties in this slab were examined by placing a 1.3 cm x 1.3 cm grid on top of the sediment slab and each grid cell was examined for roots and detritus as well as any other sources of sulfide production or reduction. Grid cell contents were compared with sulfide concentration as determined from the DGT to determine if sulfide concentration and distribution were influenced by varying levels of organic detritus in a natural system.

2.6 Laboratory Experiment

In order to determine the mechanism through which eelgrass detritus and rhizospheres influence pore-water sulfide concentration, eelgrass shoots and sediment were collected from Padilla Bay during July 2016. Aquaria were filled with Padilla Bay sediment carefully excavated and placed into the aquarium to preserve the vertical stratigraphy. Detritus pieces were cut from the oldest leaves of nearby large eelgrass shoots. From each leaf selected, 3 detritus pieces each measuring 7.6 cm in length were obtained. Eelgrass leaf detritus of varying amounts (0, 1, 2, 4, or 8) was added to the sediment on one (randomly selected) side of ten aquaria at depths of 4 cm and 11 cm (Figure 4). Live eelgrass shoots were added to five aquaria. Aquaria were placed in outdoor tanks with flowing seawater to allow for eelgrass to grow under natural light conditions. In order to prevent the sediment from sun exposure, aquaria were covered in black plastic while ensuring eelgrass shoots had access to light.

DGTs were deployed in each tank for four-hour periods once a week over four-weeks to determine how sulfide concentrations varied over time. DGTs were deployed and collected in the same order within approximately 30 minutes of each other each week to ensure that DGTs were deployed for equal lengths of time. After collection, the DGTs were deconstructed and the binding gels were subsequently scanned between two transparency sheets and analyzed as outlined in section 2.3. As with the field study sediment slabs, sediment in all aquaria was analyzed using a 1.3 cm x 1.3 cm grid for roots and detritus as well as other possible sources of sulfide production or reduction.

2.7 Statistical analysis

For the field experiment, I analyzed sulfide concentrations based on three comparisons: (1) no eelgrass vs eelgrass; (2) detritus vs no detritus; and, (3) root zone vs no root zone. The mean sulfide concentration of the entire gel was used to determine sulfide concentration differences between no eelgrass and eelgrass locations using one-way analysis of variance (ANOVA) (to account for unequal sample size). To analyze the impacts of detritus, the mean sulfide concentration of regions on each gel that were adjacent to detritus was compared to the remaining portions of the gel where no detritus was found using a paired t-test. To analyze the influence of roots, the mean sulfide concentration on each portion of gels adjacent to the root zone was compared to the remaining gel area where no roots were located using a paired t-test. The data were log transformed before analysis as a Levene's test determined the untransformed data did not exhibit homogeneity of variance.

For the laboratory experiment, I analyzed sulfide concentrations for four comparisons: (1) eelgrass vs no eelgrass; (2) detritus vs no detritus; (3) root zone vs no root zone; and, (4) detritus at 4 cm vs detritus at 11 cm (Figure 5). Analysis of co-variance (ANCOVA) was used to compare the mean sulfide concentration of the entire gel between eelgrass presence and no eelgrass presence, where the amount of added detritus was considered a covariate. For the detritus vs no detritus comparison, mean sulfide concentrations were obtained from a 2 cm x 3.5 cm block surrounding the detritus location. For a paired t-test comparison, the same size block on the opposite side of the detritus location was considered the no detritus location. The impact of the amount of detritus added was analyzed using a

regression analysis. Only the detritus and no detritus blocks at the 11-cm depth were used in the analysis to remove any influence from the root zone. An *ad hoc* pairwise t-test determined which detritus additions differed. Mean sulfide concentrations used in root zone analysis were obtained from the locations where the majority of the roots were found and the location on the opposite side of the aquarium where no roots were found. A paired t-test was used to compare sulfide concentrations between the root zone and non-root zone. To quantify the influence of depth alone on sulfide concentration, the horizontal concentration mean at each mm depth of the gel in each aquaria was calculated. A paired t-test comparing the sulfide concentrations in the non-detritus blocks at 4 cm and 11 cm was used to determine differences in depth. Repeated measures analysis of co-variance (ANCOVAR) was used to analyze the impact of week on sulfide concentrations for all aquaria. Detritus was again considered a covariate. A Levene's test determined the data did not exhibit homogeneity of variance and, therefore the data were log transformed. All statistical analyses were conducted using R statistical software (R Core Team 2016).

At the end of the aquarium experiment, large numbers of shell fragments were found in the eelgrass aquarium with 1 detritus leaf (E1). The shell likely influenced sediment chemistry by limiting the flow of pore-water throughout the sediment and therefore data were statistically analyzed both with and without this aquarium. Because the impact of detritus on sulfide was first observed during week 2 of the aquarium study, the majority of statistical analyses were conducted on data from this week.

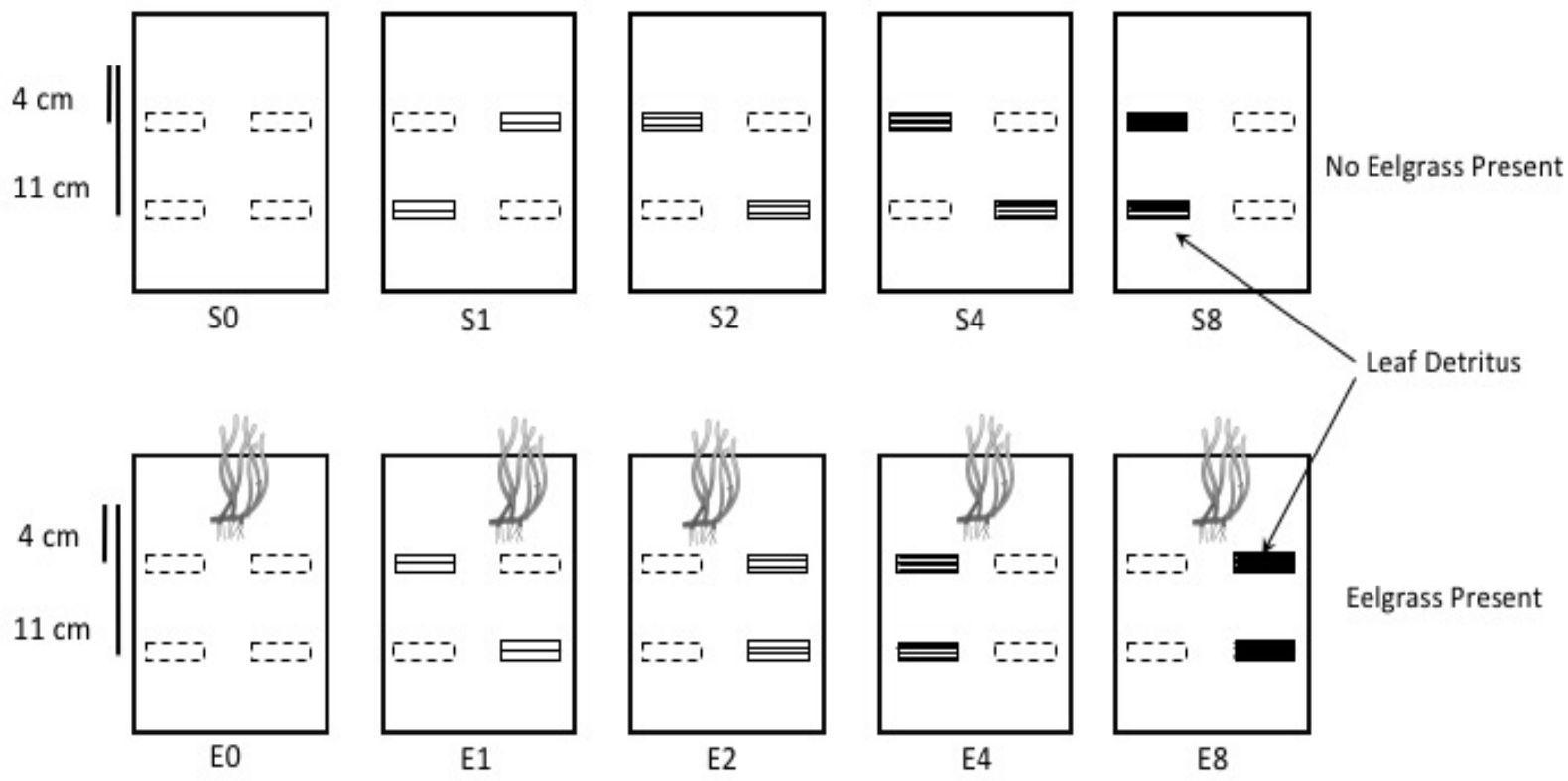

Figure 4. Diagram of the laboratory experiment. "S" indicates tanks with sediment only. "E" indicates tanks with one eelgrass shoot planted. E0 and S0 had 0 detritus leaves added, E1 and S1 had 1, E2 and S2 had 2, E4and S4 had 4 and E8 and S8 had 8. Detritus was located 4 cm and 11 cm from the sediment surface. Filled blocks indicate where leaf detritus was added and the dashed block indicates the corresponding area used in statistical analyses. Eelgrass illustration courtesy Diana Kleine, Integration and Application Network (IAN), University of Maryland Center for Environmental Science (ian.umces.edu/imagelibrary/).

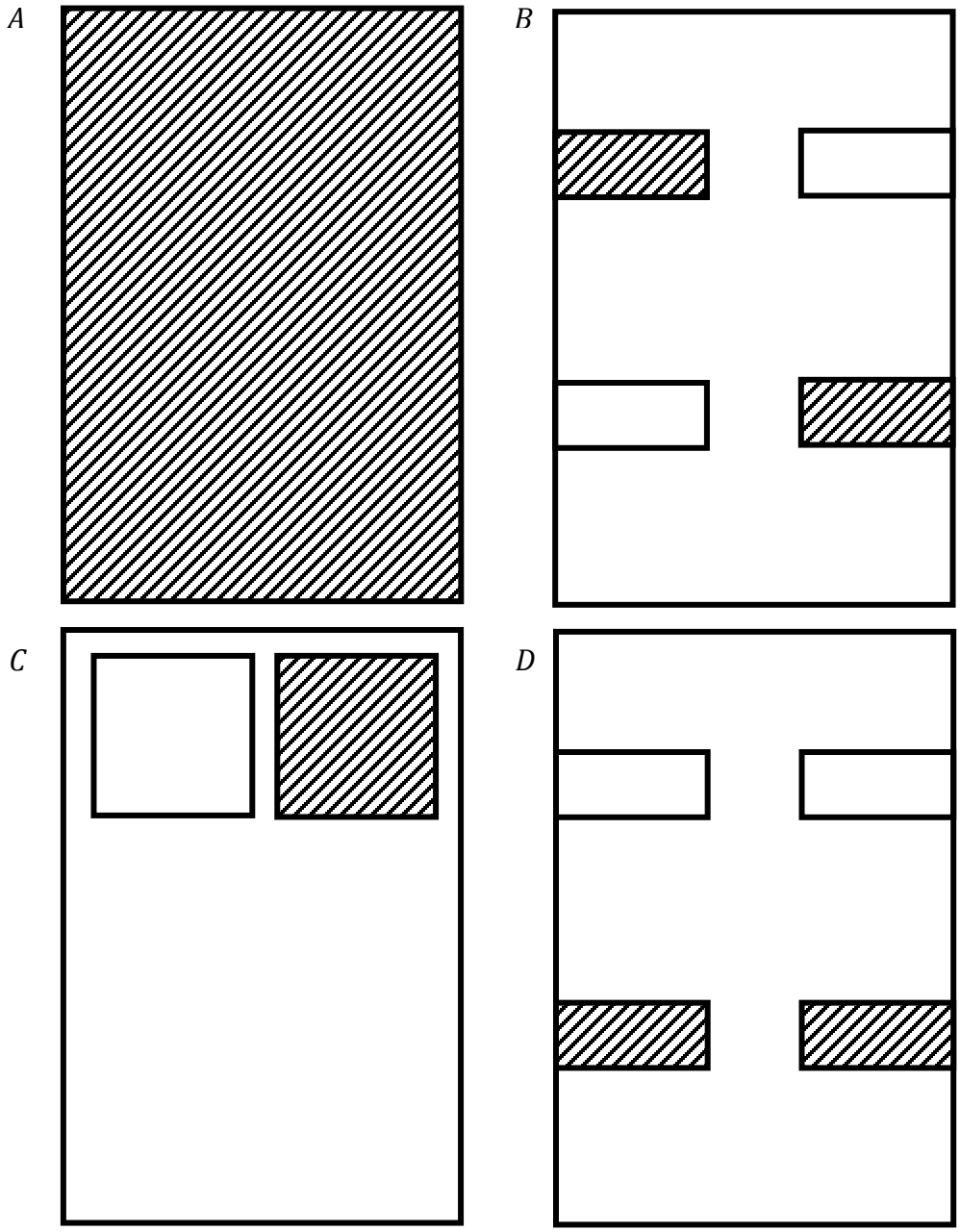

Figure 5. The locations where statistical analyses on the DGTs occurred. *A* represents the analysis of mean sulfide concentration of bare sediment vs eelgrass. *B* represents the analysis of detritus vs no detritus. *C* represents the analysis of the root zone vs no root zone. *D* represents the analysis of varying depth location.

3.0 Results

3.1 Field Study

Eight DGT probes were placed in bare patches and adjacent to eelgrass shoots throughout Padilla Bay. The DGT probes adjacent to eelgrass shoots yielded higher sulfide concentrations than those placed in bare patches (Figure 6), where the average sulfide concentration in bare patches was 0.031 mM and adjacent to eelgrass shoots was 0.048 mM (Figure 7). For instance, field probes one, two, and three, all located adjacent to multiple eelgrass shoots, had mean sulfide concentrations of 0.067 mM, 0.073 mM, and 0.043 mM, respectively. In contrast, field probes six, seven, and eight, located in bare patches yielded mean sulfide concentrations of 0.036 mM, 0.030 mM, and 0.026 mM (Figure 6). This pattern suggests that the presence of eelgrass is associated with higher sulfide concentrations when compared to unvegetated sediment (Figure 7; ANOVA, $F_{[1,6]} = 2.522$, $p = 0.16$). Eelgrass-adjacent probes four and five broke slightly from this pattern as the mean sulfide concentrations were more similar to those found in bare-patches (0.027 mM for probe four and 0.030 mM for probe five).

Additionally, the presence of detritus was associated with higher than average sulfide concentrations (Figure 7; paired t-test, $t = -13.9$, $df = 7$, $p < 0.001$). In the eelgrass-adjacent field probes contained a mean sulfide concentration of 0.069 mM. The detritus patches in eelgrass-adjacent field probes one and two, for example, yielded sulfide concentrations of 0.12 mM and 0.10 mM. This was 0.053 mM and 0.027 mM higher than the average sulfide concentrations for field probes one and two, respectively. Eelgrass-adjacent field probe three exhibited a similar pattern

wherein the mean sulfide concentration in the detritus location was 0.067 mM, resulting in 0.024 mM higher sulfide concentration compared to the corresponding average sulfide concentration (Figure 6). This pattern, though not as pronounced, was also seen in eelgrass-adjacent field probes four and five where the presence of detritus resulted in sulfide concentrations 0.002 mM higher in field probe four and 0.001 mM higher in field probe five. In bare-patch field probes six, seven, and eight, detritus was sparingly found and appeared to have little influence on sulfide concentrations (Figure 6). The mean sulfide concentrations around the detritus patches were 0.037 mM for probe six, 0.025 mM for probe seven, and 0.027 mM for probe eight (Figure 6).

In contrast, the presence of a root zone was associated with lower than average sulfide concentrations in both eelgrass-adjacent and bare-patch field probes (Figure 7; paired t-test, $t = -1.63$, $df = 7$, $p = 0.15$). All sampled bare patches, though located approximately 1 m away from any eelgrass shoot still contained small areas with roots. The root zone areas in the eelgrass-adjacent probes contained an average sulfide concentration of 0.036 mM, nearly 0.012 mM lower than the overall mean sulfide concentration (Figure 7). Eelgrass-adjacent field probes one and two contained 0.034 mM and 0.021 mM lower sulfide concentrations in the root zone compared to the overall sulfide concentration in the sediment (Figure 6). Similarly, eelgrass-adjacent field probes four and five contained sulfide concentrations 0.004 mM and 0.008 mM lower than their corresponding overall mean concentrations. Roots were found deeper in eelgrass-adjacent field probe three than in the other sediment samples, extending from 6 to 13 cm in depth. The depth of the root zone

likely led to a break in this pattern resulting in a sulfide concentration 0.010 mM higher than field probe three's respective overall concentration. Bare-patch field probes six, seven, and eight, also had lower sulfide concentrations than the overall mean in the small areas where roots were found. The mean sulfide concentration in the root zone for field probes six, seven, and eight were all 0.003 mM lower than the respective overall average sulfide concentrations (Figure 6). Overall, the presence of root zones and leaf detritus both influenced sulfide concentrations. Root zones possessed lower sulfide concentrations than detritus patches (Figure 7; paired t-test, $t = -13.8$, $df = 7$, $p < 0.001$).

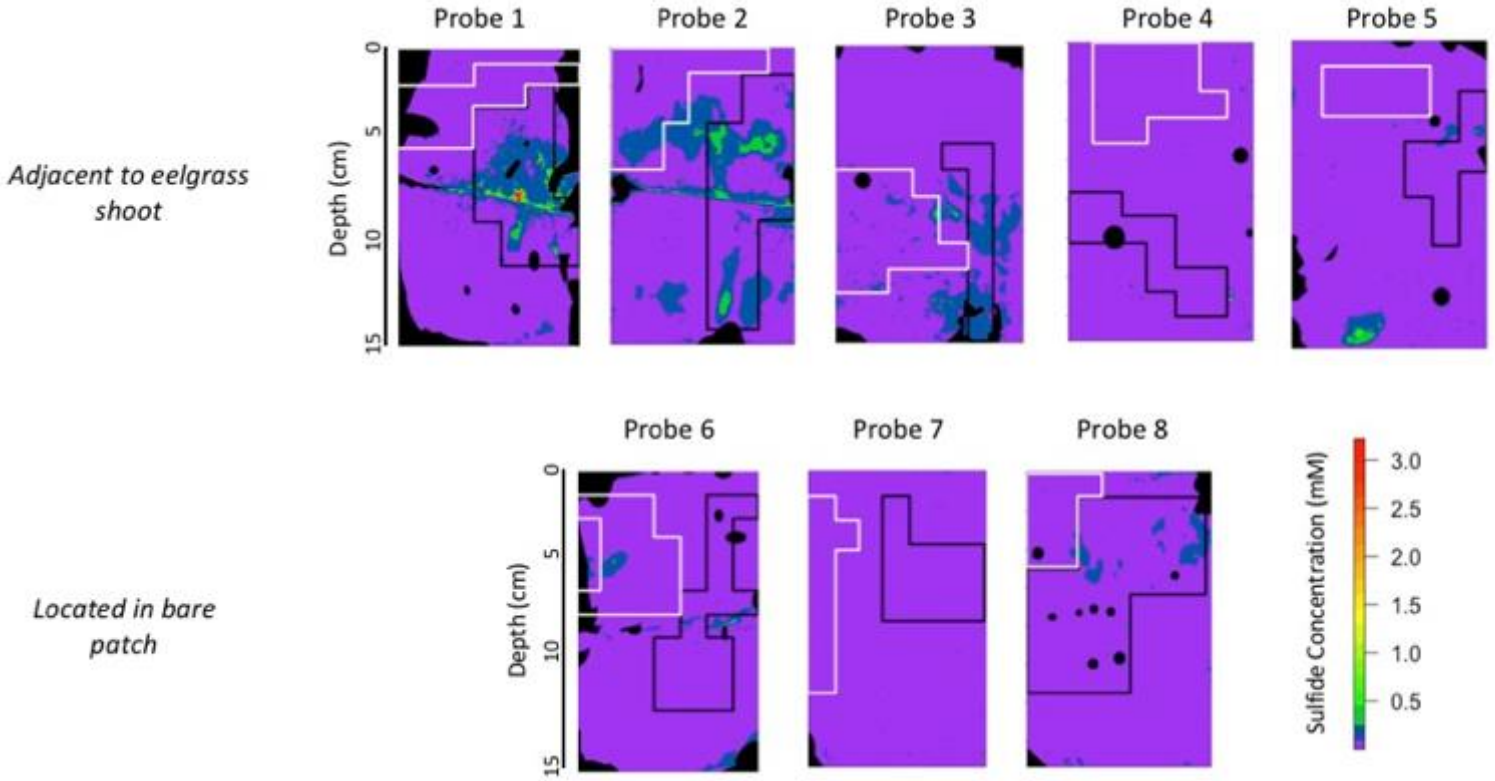

Figure 6. Field DGT probes analyzed at a 1 mm scale. White outlines indicate the combined grid cell areas where root tips were located. Roots were found in both bare patches and adjacent to eelgrass shoots. Black outlines indicate the combined grid cell areas where detritus was found. Probes 1, 2, 3, 4, and 5 were located adjacent to an eelgrass shoot, whereas probes 6, 7, and 8 were located in a bare patch.

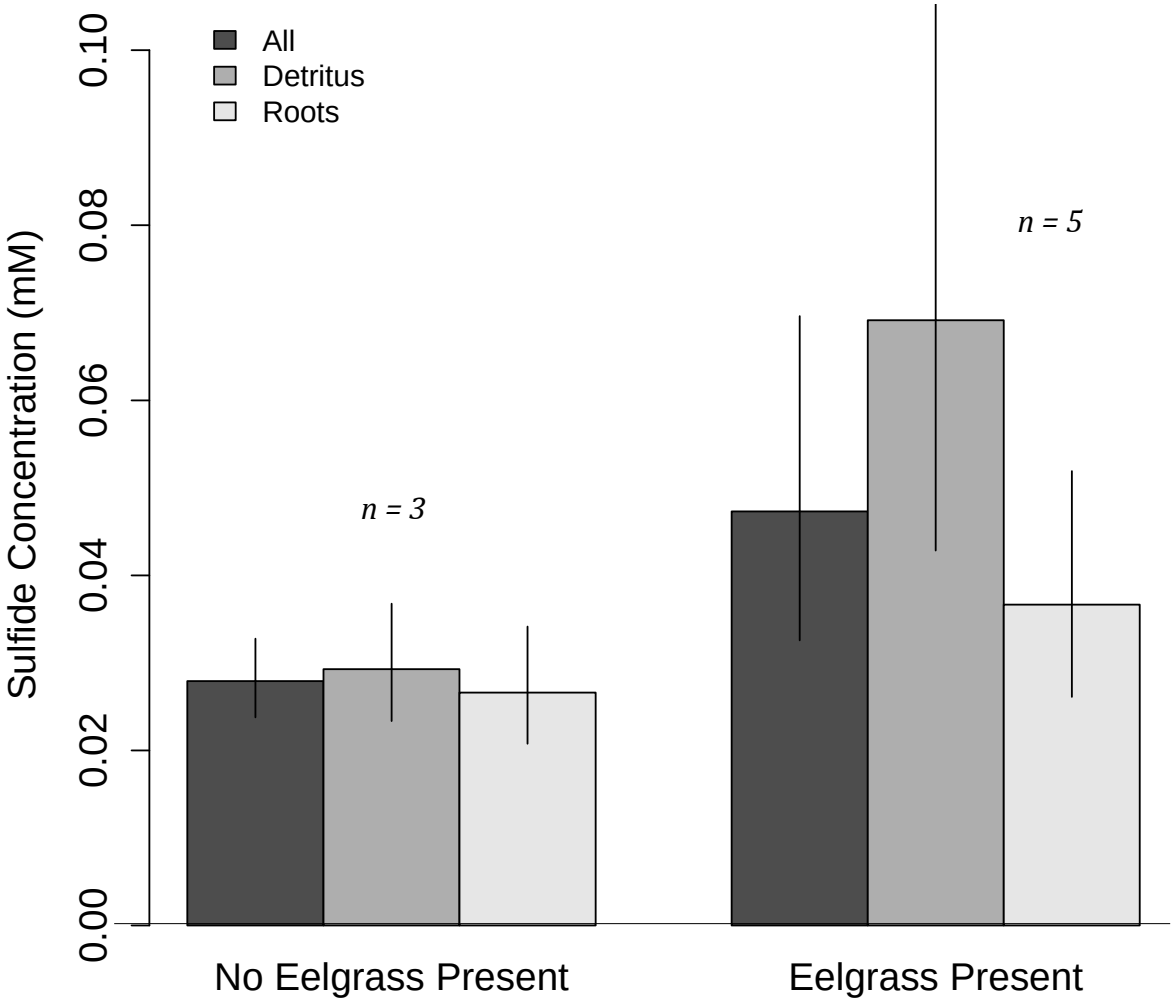

Figure 7. Analysis of field data based on the presence or absence of eelgrass. *All* refers to the mean sulfide concentration of the entire DGT sensor. *Detritus* refers to the mean sulfide concentration of areas containing detritus. *Roots* refers to the mean sulfide concentration of areas containing a root zone. Roots were found in both bare patches and adjacent to eelgrass shoots. Error bars represent 95% CI.

3.2 Laboratory Experiment

To further investigate the roles of roots and eelgrass leaf detritus in determining sediment sulfide distribution and concentration, I manipulated the quantity of leaf detritus and the presence or absence of eelgrass in aquaria fitted with DGT sensors (Figure 8). Sulfide concentrations in aquaria with and without live eelgrass generally increased from week one to week two and stabilized during week three before decreasing during week four (Figure 9; ANCOVAR, $F_{[1,7]} = 4.93$, $p = 0.001$). When not including the live eelgrass aquarium with 1 detritus leaf (E1), as it contained large amounts of shell, this difference in average sulfide concentration between aquaria with and without eelgrass did not become significant until week 4 (Figure 9; ANCOVAR, $F_{[1,34]} = 5.75$, $p = 0.018$). Nevertheless, this difference between treatments first became apparent by week 2, at which point, average sulfide levels changed little during the rest of the experiment. It appeared that sulfide concentrations generally increased with more detritus added and with the presence of live eelgrass (Figure 8; ANCOVAR, $F_{[3,21]} = 34.87$, $p < 0.001$). Furthermore, in live eelgrass aquaria, lower than average sulfide concentrations were located where the eelgrass shoot was planted (Figure 8).

During week one, sulfide concentration was low in all aquaria and localized in small patches, with the notable exception of the live eelgrass aquarium E1. In this aquarium, sulfide concentrations were widespread between 0 and 8 cm in depth across the width of the DGT and very high, reaching concentrations near 2.0 mM. This aquarium had a substantially higher sulfide concentration than any other live eelgrass aquaria with a mean sulfide concentration of 0.79 mM (Figure 8). Similarly,

in the live eelgrass aquarium with no detritus added (E0), a swath of high sulfide concentration reaching 2.5 mM was found in a localized area on the right side of the DGT from 7 to 12 cm depth. In the remaining aquaria, the highest sulfide concentrations, averaging 0.5 mM, were localized where leaf detritus was added at 11 cm in depth regardless if eelgrass was present or not. There was very little difference in sulfide concentration between aquaria with and without live eelgrass shoots during week one (Figure 8; ANCOVA, $F_{[1,38]} = 0.058$, $p = 0.809$).

During week two, average sulfide concentration in all aquaria increased relative to week one concentrations. Though sulfide concentrations increased for all aquaria, concentrations were higher in aquaria with live eelgrass compared to aquaria without live eelgrass (Figure 8; ANCOVA, $F_{[1,38]} = 6.78$, $p = 0.010$). Live eelgrass aquaria, regardless of the detritus quantity added, reached peak sulfide concentrations and high sulfide concentrations spanned large portions of the DGT (Figure 8). For instance, the live eelgrass aquarium E0, had high sulfide concentrations from 4 cm to 14 cm in depth spreading across the width of the DGT. This pattern was also seen in the live eelgrass aquarium E1 as this it had extremely high sulfide concentrations, ranging from 2.0 to 2.5 mM found from 2 to 13 cm in depth stretching to both sides of the DGT. Furthermore, the live eelgrass aquarium with 8 detritus leaves (E8), contained a large patch of sulfide on the right side of the DGT where the detritus leaves were added. The aquaria without live eelgrass shoots also appeared to reach peak sulfide concentrations, though localized around the areas where detritus was added (Figure 8). This is easily seen in the aquarium without live eelgrass with eight detritus leaves added (S8). Here, two patches of

sulfide appeared in the same locations where leaf detritus was added on the left side at 4 cm and 11 cm in depth The patch at the deeper depth was larger and had higher sulfide concentrations than the surface patch. This pattern was also reflected in the aquarium without eelgrass and one detritus leaf added (S1), however, the patch of detritus nearer the surface resulted in higher sulfide concentrations than the detritus added at depth. The aquarium without eelgrass and four detritus leaves added (S4) varied slightly from this pattern. High sulfide concentrations, reaching above 2 mM, spanned the lower half of the gel, from 10 cm to 15 cm. The highest sulfide concentration, however, was located near the 11-cm detritus addition.

The difference between sulfide concentrations for aquaria with and without live eelgrass persisted through week 3 (ANCOVA, $F_{[1,38]} = 4.61$, $p = 0.033$) and week 4 (ANCOVA, $F_{[1,38]} = 4.70$, $p = 0.032$). Generally, sulfide concentrations in all aquaria during week three was similar to week two, though, spanning a larger extent (Figure 8). For example, sulfide concentrations in the live eelgrass aquarium with 2 detritus leaves (E2) remained highest around the detritus addition locations, though the presence of sulfide spanned from 4 cm to 14 cm in depth. During week 4, sulfide concentrations in the live eelgrass aquaria decreased but continued to dissipate throughout the gel. For instance, live eelgrass aquaria E0 and E1 both had sulfide concentrations near 2.0 mM during week two yet during week four sulfide concentrations decreased to approximately 0.5 mM. In aquaria without live eelgrass, sulfide concentrations dropped substantially during week four (Figure 8). Aquaria without live eelgrass and with 0 (S0), 1 (S1), and 2 (S2) detritus leaves contained extremely low sulfide concentrations hovering near 0 mM.

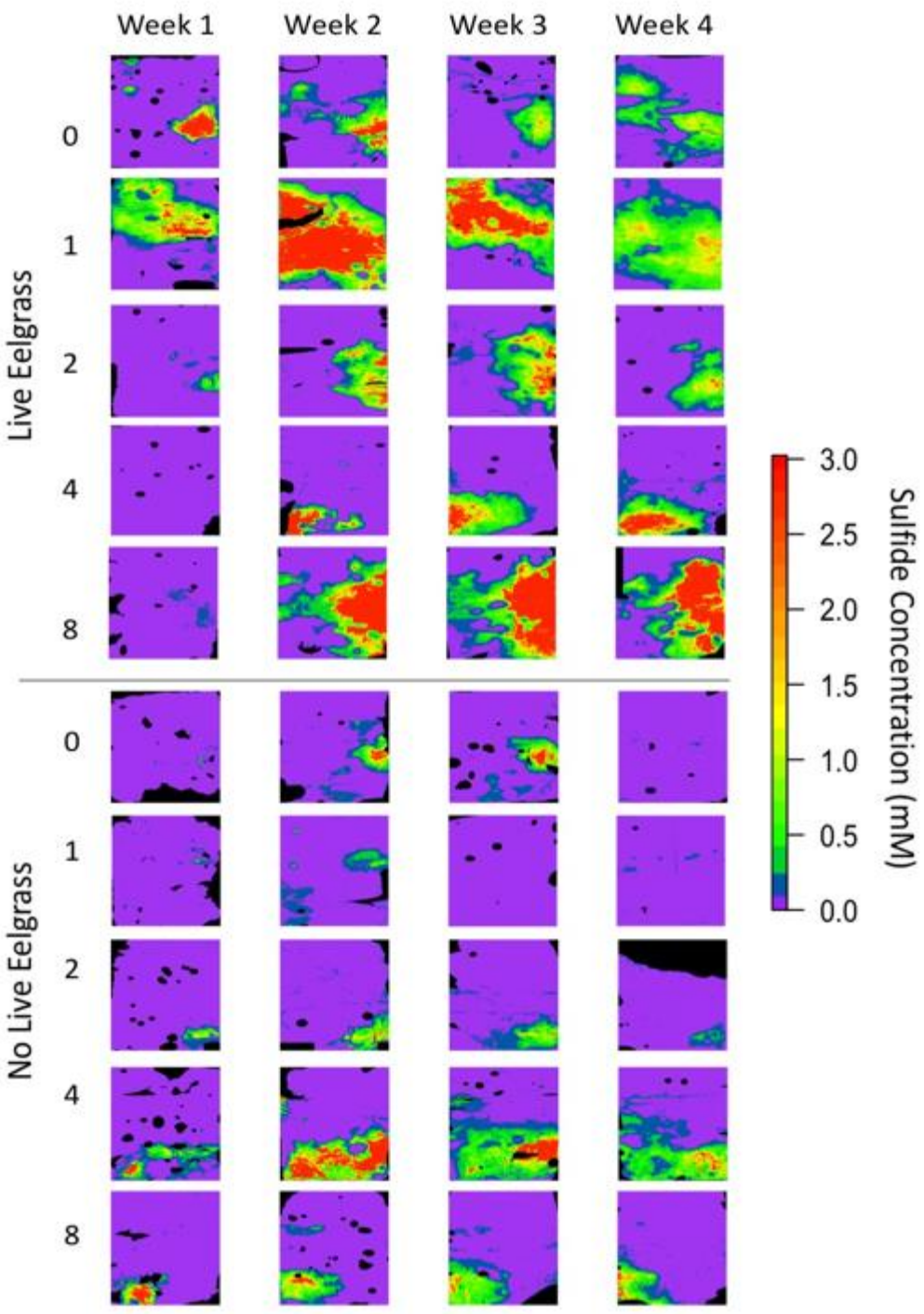

Figure 8. Comparison of all aquaria over each week of the laboratory experiment. 0, 1, 2, 4, and 8 indicate the number of detritus leaves added at each depth. *Live eelgrass* refers to the aquaria with one eelgrass shoot planted including E0, E1, E2, E4, and E8. *No live eelgrass* refers to the aquaria without an eelgrass shoot planted including S0, S1, S2, S4, and S8.

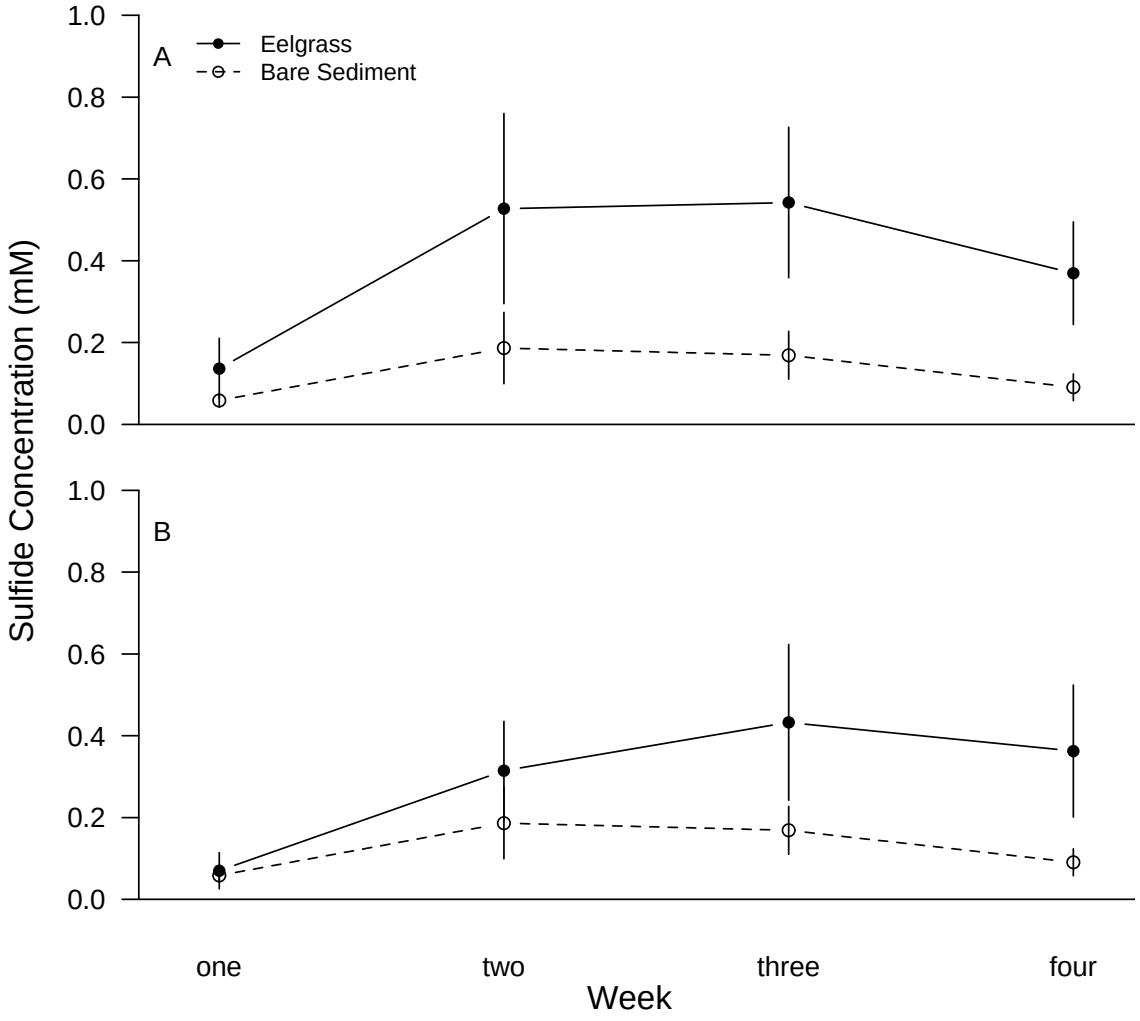

Figure 9. Mean sulfide concentrations for aquarium with and without live eelgrass shoots over each week. *A* represents analysis with the full data set while *B* represents analysis excluding E1. Error bars represent ± 1 SE with week as a replicate

3.3 Presence of Live Eelgrass Shoots

Aquaria containing live eelgrass shoots generally had higher average sulfide concentrations than the aquaria containing bare sediment even after considering the variation of added detritus (ANCOVAR, $F_{[1,8]} = 10.02$, $p = 0.013$). Aquaria containing bare sediment had low pore-water sulfide concentrations with a mean sulfide concentration of 0.13 mM, whereas, the mean sulfide concentration for aquaria containing eelgrass was 0.40 mM (Figure 8). The presence of eelgrass increased the average sulfide concentration by 0.27 mM.

When analyzing all aquaria and taking into consideration week and quantity of detritus added as covariates, the presence of live eelgrass shoots increased sulfide concentrations (ANCOVA, $F_{[1,34]} = 9.828$, $p = 0.003$), with covariate adjusted means of 0.126 ± 0.060 mM for bare sediment aquaria and 0.394 ± 0.060 mM for live eelgrass aquaria. When excluding aquarium E1, the presence of eelgrass again increased sulfide concentrations (ANCOVA, $F_{[1,30]} = 6.05$, $p = 0.012$) resulting in covariate adjusted means of 0.135 ± 0.040 mM for bare sediment and 0.284 ± 0.045 mM for eelgrass aquaria. However, when excluding E1, the amount of detritus added also influenced sulfide concentration (ANCOVA, $F_{[1,30]} = 10.187$, $p = 0.003$).

3.4 Detritus Additions

To determine the effect of differing amounts of detritus on sulfide concentrations, only the deeper depths were analyzed to reduce the possible effects of eelgrass roots. Detritus additions were converted to Ash-Free Dry Weight (AFDW) given that one piece of added detritus averaged 0.0103 g AFDW. More

detritus added yielded higher sulfide concentrations using the full week 2 data (Figure 10; paired t-test, $t = 2.69$, $df = 9$, $p = 0.025$) and when excluding aquarium E1 (Figure 10; paired t-test, $t = 2.90$, $df = 8$, $p = 0.019$). Using a pairwise t-test to compare the different detritus additions, the greatest increase in sulfide concentrations was seen between the 0 and 8 piece treatments (pairwise t-test, $p < 0.001$). When not including aquarium E1, large differences in sulfide concentrations were seen between 0 and 4 pieces (pairwise t-test, $p = 0.032$), between 0 and 8 pieces (pairwise t-test, $p < 0.001$), between 1 and 8 pieces (pairwise t-test, $p = 0.001$) and between 2 and 8 pieces (pairwise t-test, $p = 0.029$).

3.5 Rhizosphere Presence

The sulfide concentration around the rhizosphere was an order of magnitude lower than the adjacent non-root zone with a mean of 0.048 mM for the rhizosphere and a mean of 0.48 mM for the non-root zone (Figure 11). Using week 2 values, the presence of roots reduced sulfide concentrations compared to areas where no roots were present (paired t-test, $t = 3.15$, $df = 4$, $p = 0.035$). When excluding E1, roots still influenced the sulfide concentrations within the sediment by lowering concentrations nearer the root zone (paired t-test, $t = 2.62$, $df = 3$, $p = 0.079$).

3.6 Depth Analysis

The depths at which sulfide concentrations were highest varied considerably across aquaria (Figure 12). Aquaria E1 and E8 had high concentrations across all depths, declining toward the surface and at 15 cm depth. For E0, S0, E2 and S4,

peaks of high sulfide concentration are found throughout different depths but the largest peak in sulfide concentration occurred around 10 cm. In S2, E4, and S8, sulfide concentrations peaked at approximately 13 cm. S2 contained low concentrations throughout with a minimal spike in sulfide occurring around 6 cm in depth (Figure 12). On week 2, deeper sediment accounted for higher sulfide concentrations than surface sediment (paired t-test, $t = 3.52$, $df = 19$, $p = 0.002$). When excluding E1, this difference was still apparent (paired t-test, $t = 3.11$, $df = 17$, $p = 0.006$).

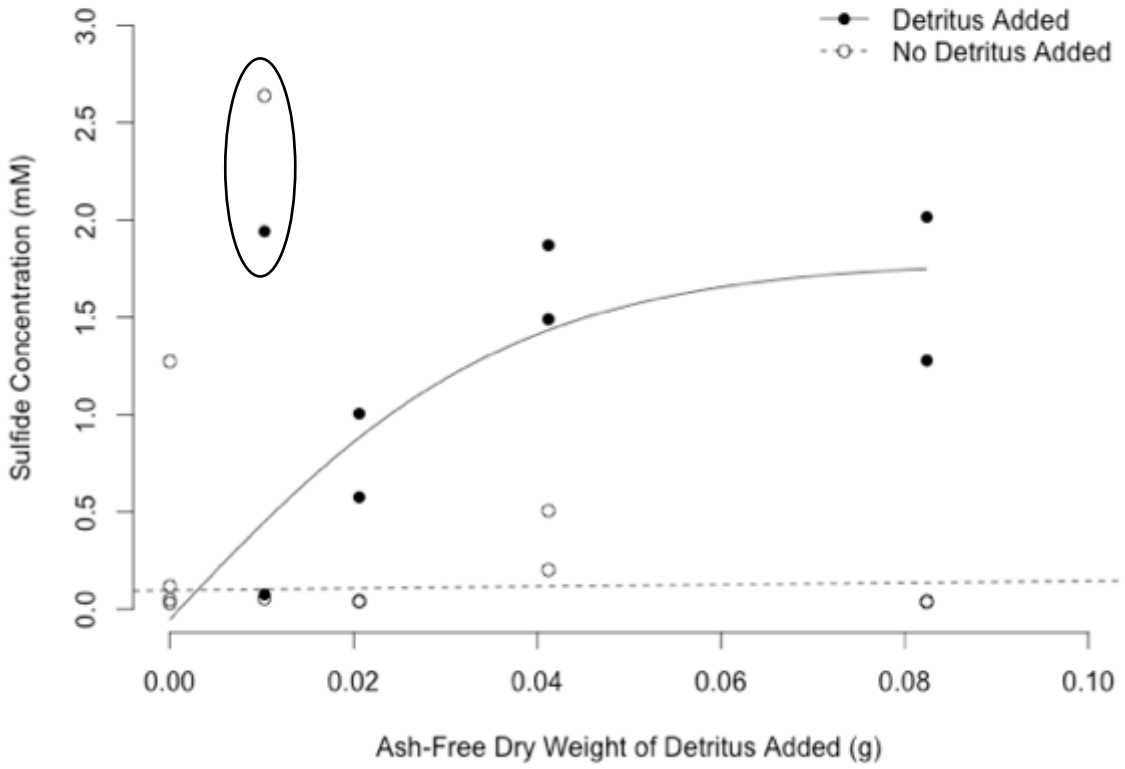

Figure 10. Average sulfide concentrations for locations with and without detritus leaves added at 11 cm depth versus mass of detritus added. The regression lines show the relationship between sulfide concentration and mass of detritus added when not including aquarium E1. The circled points indicate the values obtained from aquarium E1.

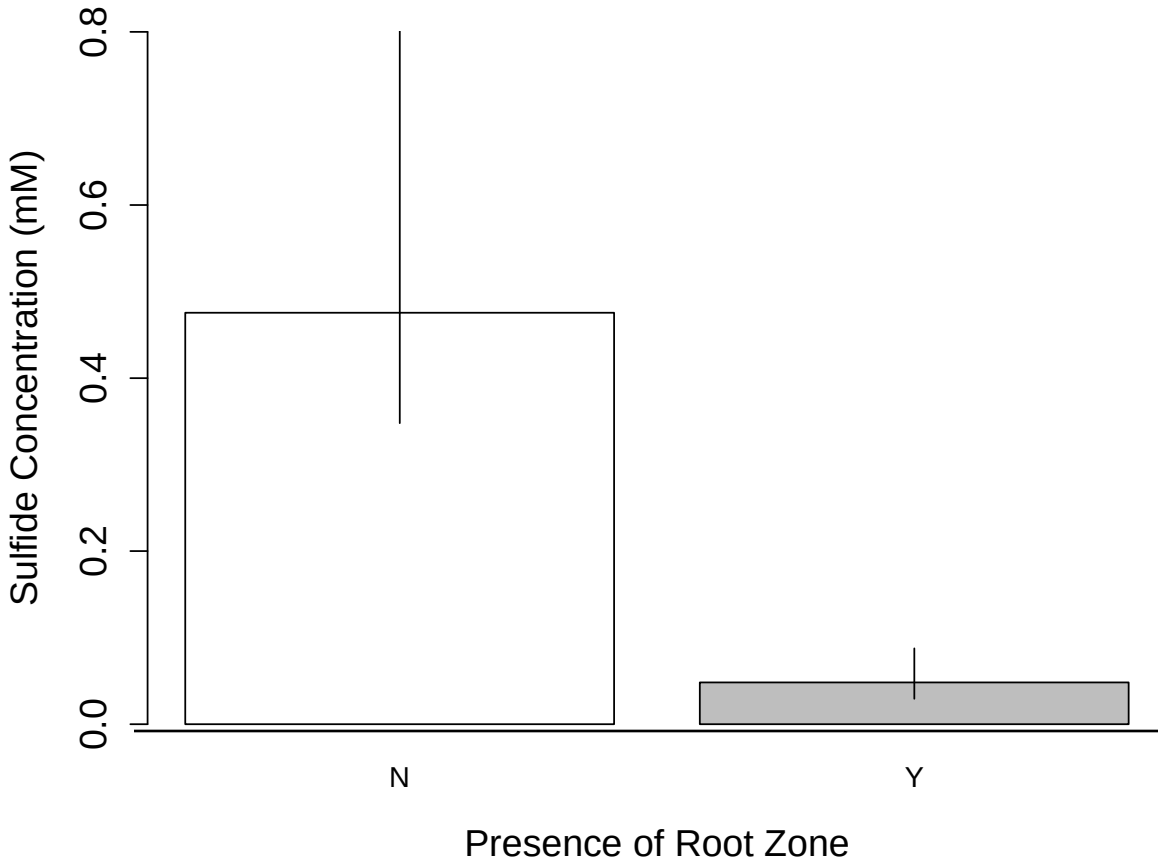

Figure 11. Comparison of sulfide concentrations for eelgrass aquaria in locations with and without roots. The root zone was defined as the area where the majority of root tips were located. Sulfide concentrations from the no root zone category were determined by using an area of the same size as the root zone on the opposite side of the DGT. Error bars indicate 95% CI (n=5) after back transforming data.

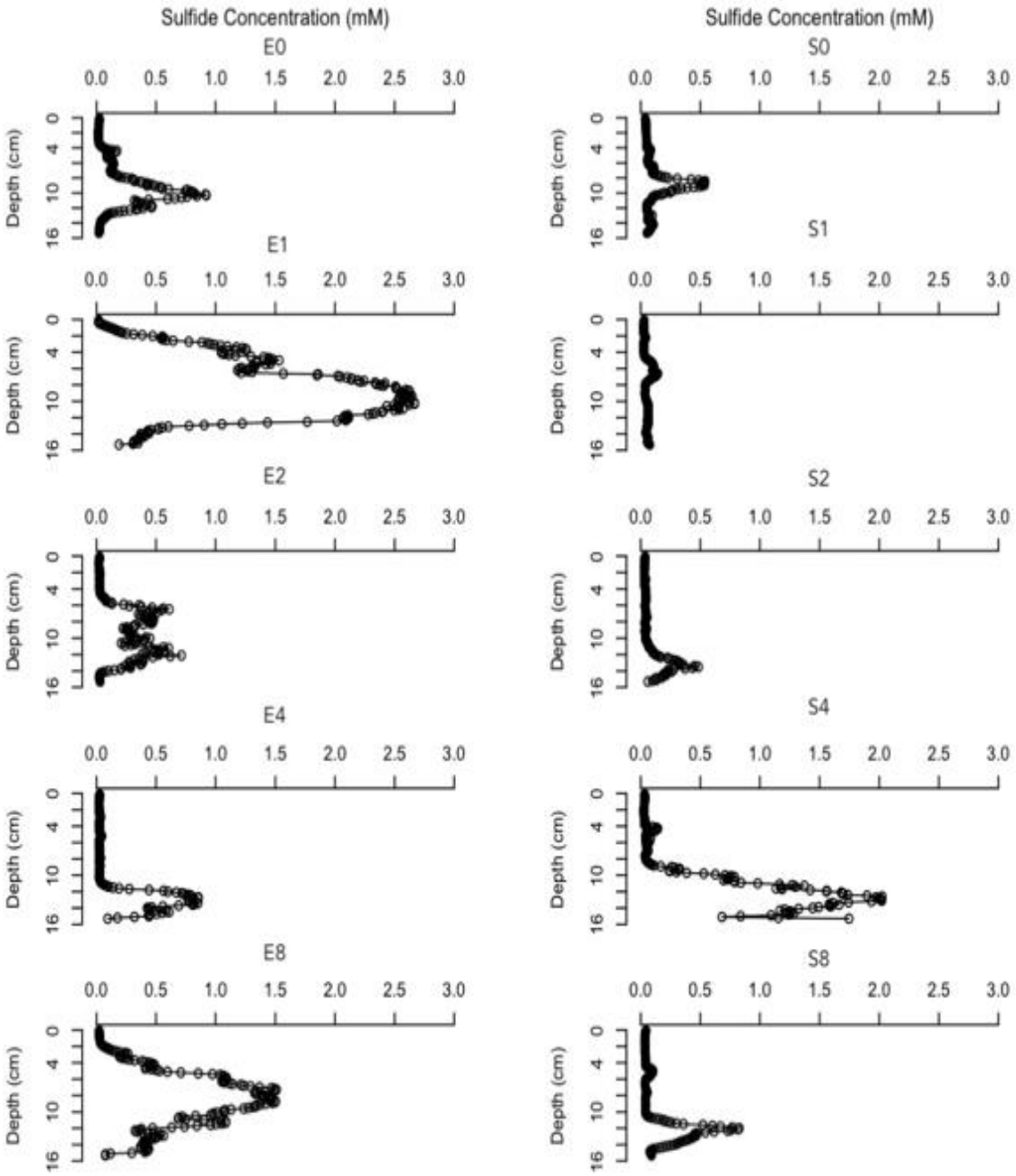

Figure 12. Analysis of sulfide concentrations by depth for week two for all aquaria. The x-axis represents horizontally averaged sulfide concentrations in mM at 1 mm scales.

4.0 Discussion

Often, the enhancement of pore-water sulfide in sediment inhabited by eelgrass has been attributed to an input of organic matter through the leakage of dissolved organic carbon from roots (Wetzel and Penhale 1979; Blaabjerg and Finster 1998; Holmer et al. 2001). Furthermore, Boschker et al. (2000) previously determined that the input of organic matter from seagrasses had little impact on initializing anaerobic mineralization, furthering the notion that the input of organic matter is typically from dissolved organic carbon. However, I determined that sulfide concentrations were highest in sediment surrounding leaf detritus in both the field and in manipulative laboratory experiments and that sulfide concentration increased with increasing mass of added detritus. Furthermore, the presence of eelgrass was found to simultaneously increase the sulfide concentrations away from the root zone and decrease concentrations nearer the root zone.

4.1 Live Eelgrass Presence

The presence of eelgrass in both the field and laboratory led to higher pore-water sulfide concentrations compared to unvegetated sediments. This corroborates previous studies which indicate higher pore-water sulfide concentrations are found in eelgrass inhabited sediments (Holmer and Nielson 1997; Blaabjerg and Finster 1998; Blaabjerg et al. 1998) despite the risk of sulfide toxicity to eelgrass (Bagarinao 1992; Holmer and Bondgaard 2001; Koch and Erksine 2001; Pedersen et al. 2004; Borum et al. 2005, 2014; Korhonen et al. 2012; Lamers et al. 2013). Furthermore, sulfate reduction rates have been found to be up

to 20 times greater in sediments containing *Z. marina* roots and rhizomes than in sediments with the roots and rhizomes removed (Blaabjerg and Finster 1998; Blaabjerg et al. 1998; Welsh 2000). Likewise, in *Zostera noltii* meadows, as well as in salt marshes, sulfate reduction is higher in vegetated than unvegetated sediments (Nielsen et al. 2001). In the current study, the difference in sulfide concentrations between eelgrass-inhabited sediments and unvegetated sediments in the field study was not as great as seen in the laboratory study. This could be due to reduced power water exchange in the aquaria compared to the field site, which is exposed to greater tidal current velocities and periodic subaerial exposure. (Padilla Bay NERR 2011). Nevertheless, eelgrass in both environments increased sulfide concentration within the surrounding sediments. This might occur via several mechanisms. Eelgrass inhabited sediments contain a large population of sulfate-reducing bacteria (Cifuentes et al. 2000; Jensen et al. 2007), leading some to believe there is a mutualistic association between seagrasses and sulfate-reducing bacteria (Welsh 2000; Smith et al. 2004). The population size of sulfate-reducing bacteria in marine vegetated sediments has been observed to be an order of magnitude greater than in unvegetated sediments (Jorgensen and Bak 1991). Thus, the high sulfide concentrations observed in the laboratory study could be due to a large sulfate-reducing bacterial population. However, this reasoning does not provide an explanation for the strong spatial variation in sulfide concentration within each aquaria. Other studies have suggested the leakage of dissolved organic carbon from roots could increase sulfide concentrations (Wetzel and Penhale 1979; Blaabjerg and Finster 1998; Holmer et al. 2001) as could the presence of detritus (Boschker et

al. 2000). Both could account for the variation in sulfide distribution and concentration among and within aquaria as both roots and detritus are spaced heterogeneously throughout the sediment.

4.2 Detritus Effect

The dramatic effect of eelgrass detritus on sulfide was particularly evident in Probe 2 from the field study where the region of high sulfide concentration clearly followed the location of a large buried eelgrass leaf. In the laboratory study, the addition of eelgrass detritus also increased pore-water sulfide concentrations. A similar pattern was observed when non-dissolved organic matter was added in *Posidonia australis* meadows which led to more reducing sediments (Fraser et al. 2016). In the study by Fraser et al. (2016), however, the organic matter, a ground mixture of *P. australis* leaf and fibrous material, was homogenously added into the sediment compared to localized placement as done here. The addition of sucrose in *Z. marina* and *Cymodocea nodosa* meadows also increased sulfide concentrations (Terrados et al. 1999). Similarly, anthropogenic wood waste as a source of organic matter in marine sediment, has been found to increase sulfide to extremely high concentrations leading to complete loss of eelgrass (Elliot et al. 2006). Also, organic enrichment leading to high sulfide concentrations has been linked to the massive die-off of the tropical seagrass *Thalassia testudinum* (Carlson et al. 1994). Furthermore, eutrophication, though widely thought of as limiting light availability, also has the capability of increasing sulfide concentrations as the increase in phytoplankton growth would subsequently lead to an increase in organic matter

(Burkholder et al. 2007). However, as shown in this study, the effects of increased sulfide concentrations appear to be localized with high concentrations immediately in the vicinity of added organic matter. Previous studies where organic matter was intentionally added in seagrass meadows (Terrados et al. 1999; Fraser et al. 2016) did not indicate that sulfide concentrations increased primarily around the organic matter addition. It is likely, though, that the researchers were unable to observe this pattern using their sulfide measuring technique (redox potential).

4.3 Rhizosphere Effect

In the field, the presence of a root zone contributed to lower mean sulfide concentrations than in sediment without a root zone. Furthermore, in the laboratory study, to the root zone had approximately an order of magnitude lower sulfide concentrations than the non-root zone. Even though the oxic zone around *Z. marina* root tips only extends ~2-3 mm beyond the root tip, clusters of root tips create a much larger oxic area extending up to 8 mm along the root (Frederiksen and Glud 2006). In multiple eelgrass aquaria, the impact on sulfide concentration from clustering root tips was noticeable even when examined on a larger scale of 1.7 cm^2. However, the plants used in previous work by Frederiksen and Glud (2006) were smaller (26.6+/- 5.2 cm) than plants used in this study (~40-50 cm) suggesting that the root zone would be larger leading to a bigger oxic area. High oxygen production around the root zone is also found in other seagrasses, such as *Zostera muelleri* (Koren et al. 2015). Oxygen leakage from the roots, however, is highly localized and maintains its largest impact nearest the root tip (Koren et al. 2015). This was

evident in this study as well. For instance, the top corner of aquarium E1, where the eelgrass shoot was planted, had lower sulfide concentrations each week than other parts of the sediment as well as in E8, where high sulfide concentrations were found throughout the DGT except in the location where the eelgrass shoot was planted. However, *Z. marina* roots typically have limited oxygen leakage from their roots in comparison to other seagrasses and therefore have a low sulfide threshold of < 0.5 mM (Pedersen and Kristensen 2015). Here, the mean sulfide concentration in the aquaria containing eelgrass shoots was 0.40 mM indicating that the eelgrass roots were not in danger of exposure to the sulfide threshold despite being surrounded by high sulfide concentrations > 1 mM.

4.4 Depth Effect

Sulfate reduction typically occurs at depth in the sediment due to microbial preference for more energetically favorable electron acceptors closer to the surface (Burdige 2006; Mitsch and Gosselink 2007). In field sediments, the highest sulfide concentrations typically occurred from approximately 8 to 12 cm in depth, which is fairly typical of coastal sediments (Burdige 2006). The wide depth range of sulfide in vegetated aquaria seems typical for eelgrass-inhabited sediments especially during summer months when sulfide can be found even near the sediment surface (Frederiksen et al. 2006). A wide spatial variation in sulfide concentrations has been noted in other seagrass species, such as *T. testudinum,* as well (Chambers et al. 2001). Furthermore, a study on *Zostera noltii* inhabited sediments suggested that the top few centimeters of vegetated sediment yielded nearly twice the sulfide

production compared to unvegetated sediments (Isaken and Finster 1996). This further confirms that the spatial distribution of sulfide in seagrass vegetated sediments is far wider than in unvegetated sediments due to the many factors leading to higher sulfide production.

4.6 For Further Study

As DGTs calculate total pore-water sulfide (Teasdale et al. 1999), measuring pH to obtain quantities of H_2S and HS^- would aid in the understanding of sulfide toxicity, which is higher for H_2S. Excess quantities of H_2S is typically the cause of seagrass growth limitation and die-offs (Carlson et al. 1994; Borum et al. 2014), and determining the concentrations of various sulfide species would improve the usefulness of the DGT method. Furthermore, it is possible to obtain iron data simultaneously as sulfide data from DGTs (Pagès et al. 2012), however, it was found to not be effective when sulfide concentrations were above 0.5 mM.

Using DGTs can also improve understanding of differences in sediment chemical kinetics among various seagrass species. To my knowledge, DGTs have yet to be used as a comparison tool to understand the spatial variation of sulfide among root zones of different species, even though they present an easy opportunity to do so. Furthermore, extended understanding of light impacts and seasonality on sulfide concentrations in seagrass sediments could also be studied using the DGT method.

4.7 Conclusion

The presence of eelgrass can simultaneously increase the sulfide concentrations away from the root zone and decrease concentrations within the root zone though this would have been difficult to determine without the use of DGTs. Furthermore, eelgrass detritus was found to be an important contributor to sulfide production within eelgrass beds. This is significant because eelgrass leaf detritus can be easily transported by currents and has the ability to accumulate in areas far from eelgrass beds. Thus, eelgrass could potentially influence the chemistry of sediments far from the beds they inhabit. Eelgrass could live in sediment with moderately high sulfide concentrations as long as they are able to photosynthesize and oxidize sulfide within the root zone. This indicates that areas previously believed to be inadequate for eelgrass restoration due to moderately high levels of sulfide could potentially be restored by planting adult eelgrass shoots as long as other stressors such as low light are controlled.

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APPENDIX

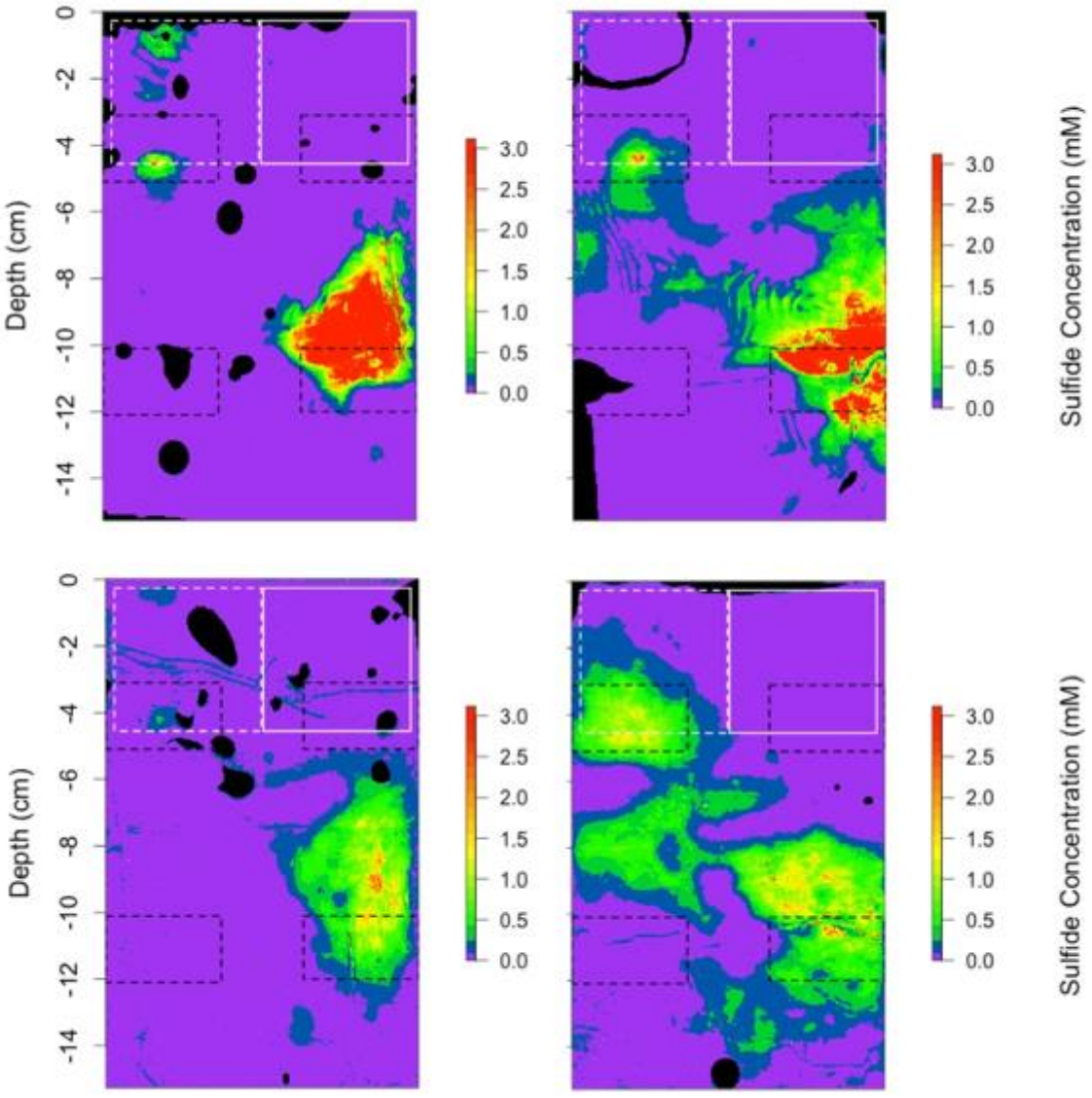

Figure A1. DGT sulfide concentrations for aquarium with live eelgrass and 0 detritus leaves (E0). The dotted black rectangles show the areas calculated as controls for detritus. The solid white rectangle shows the extent of the root zone. The dotted white rectangle represents the area calculated as the no root zone. Week 1 through week 4 are shown from top left to bottom right.

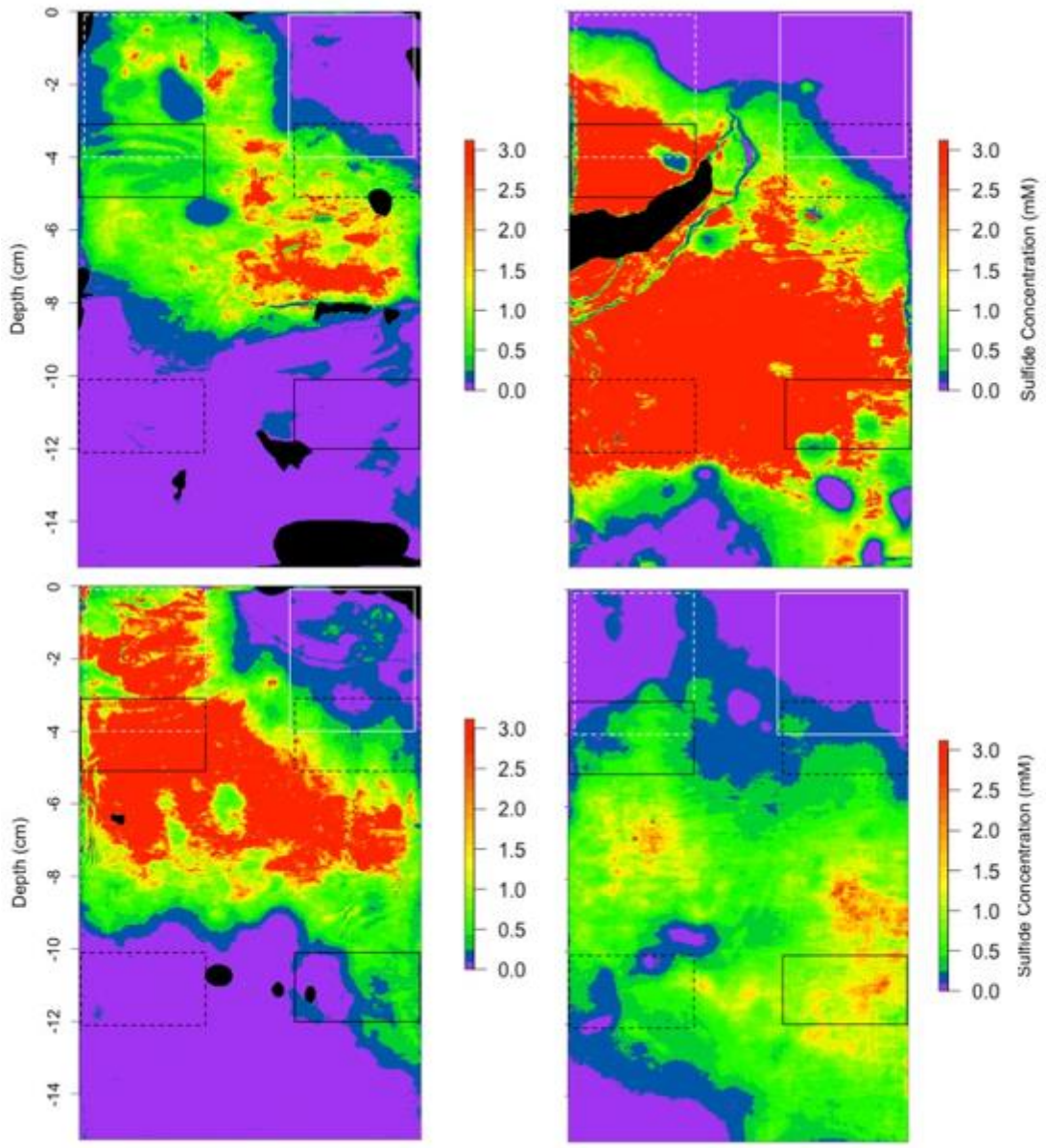

Figure A2. DGT sulfide concentrations DGT for aquarium with live eelgrass and 1 detritus leaf (E1). The dotted black rectangles show the areas calculated as controls for detritus. The solid black rectangles show the areas where sulfide concentrations from detritus were calculated. The solid white rectangle shows the extent of the root zone. The dotted white rectangle represents the area calculated as the no root zone. Week 1 through week 4 are shown from top left to bottom right.

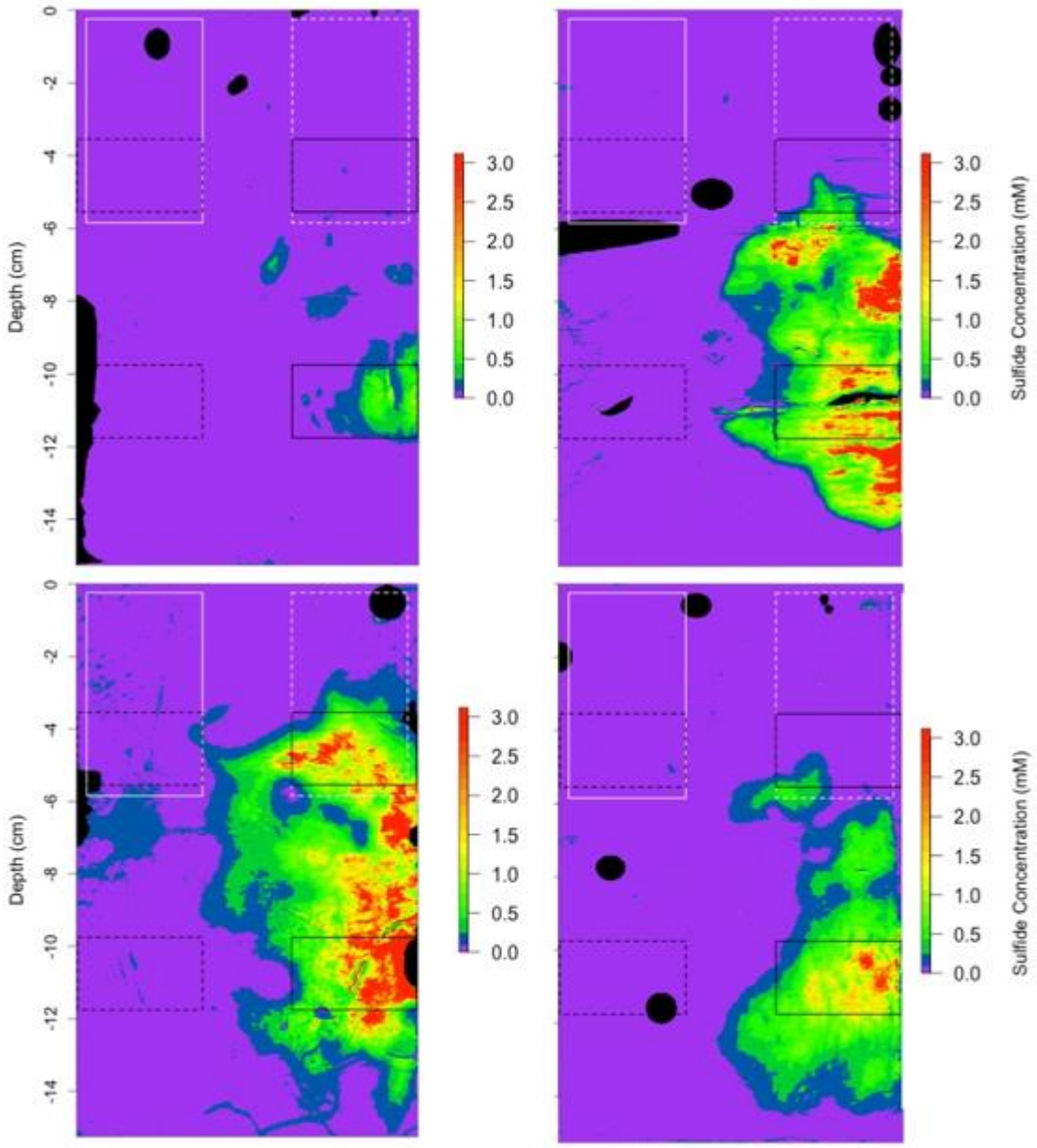

Figure A3. DGT sulfide concentrations DGT for aquarium with live eelgrass and 2 detritus leaves (E2). The dotted black rectangles show the areas calculated as controls for detritus. The solid black rectangles show the areas where sulfide concentrations from detritus were calculated. The solid white rectangle shows the extent of the root zone. The dotted white rectangle represents the area calculated as the no root zone. Week 1 through week 4 are shown from top left to bottom right.

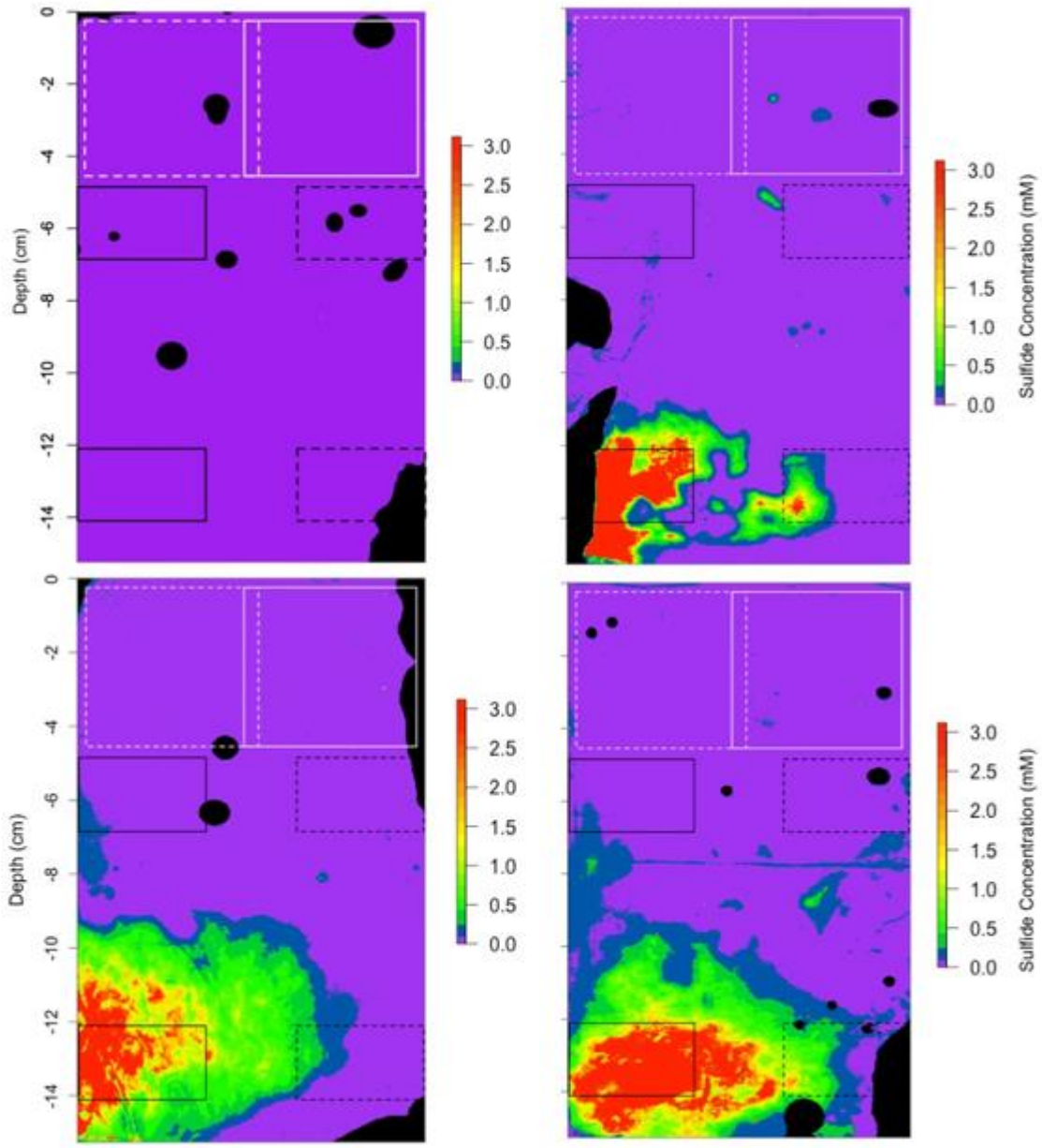

Figure A4. DGT sulfide concentrations DGT for aquarium with live eelgrass and 4 detritus leaves (E4). The dotted black rectangles show the areas calculated as controls for detritus. The solid black rectangles show the areas where sulfide concentrations from detritus were calculated. The solid white rectangle shows the extent of the root zone. The dotted white rectangle represents the area calculated as the no root zone. Week 1 through week 4 are shown from top left to bottom right.

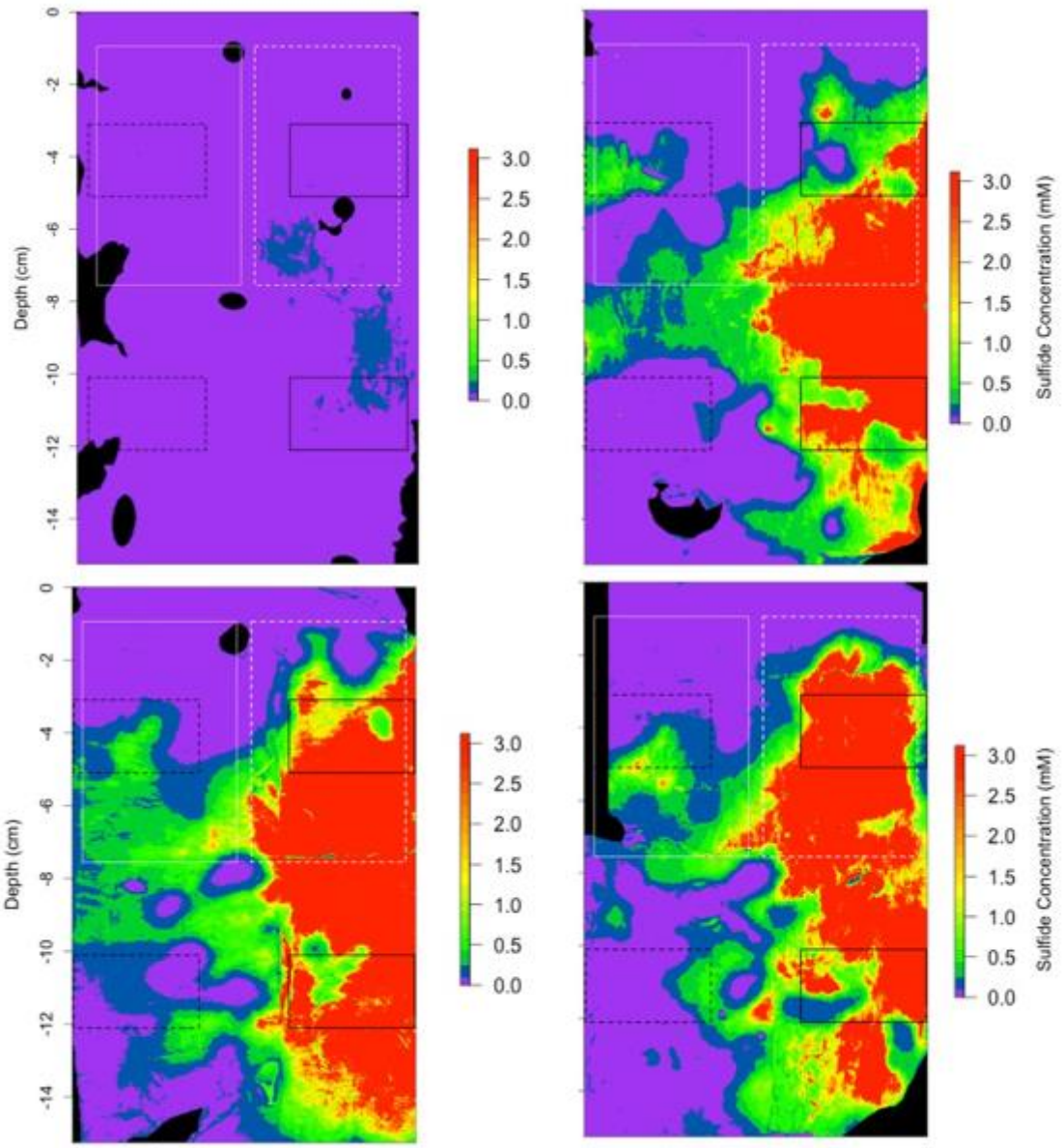

Figure A5. DGT sulfide concentrations DGT for aquarium with live eelgrass and 8 detritus leaves (E8). The dotted black rectangles show the areas calculated as controls for detritus. The solid black rectangles show the areas where sulfide concentrations from detritus were calculated. The solid white rectangle shows the extent of the root zone. The dotted white rectangle represents the area calculated as the no root zone. Week 1 through week 4 are shown from top left to bottom right.

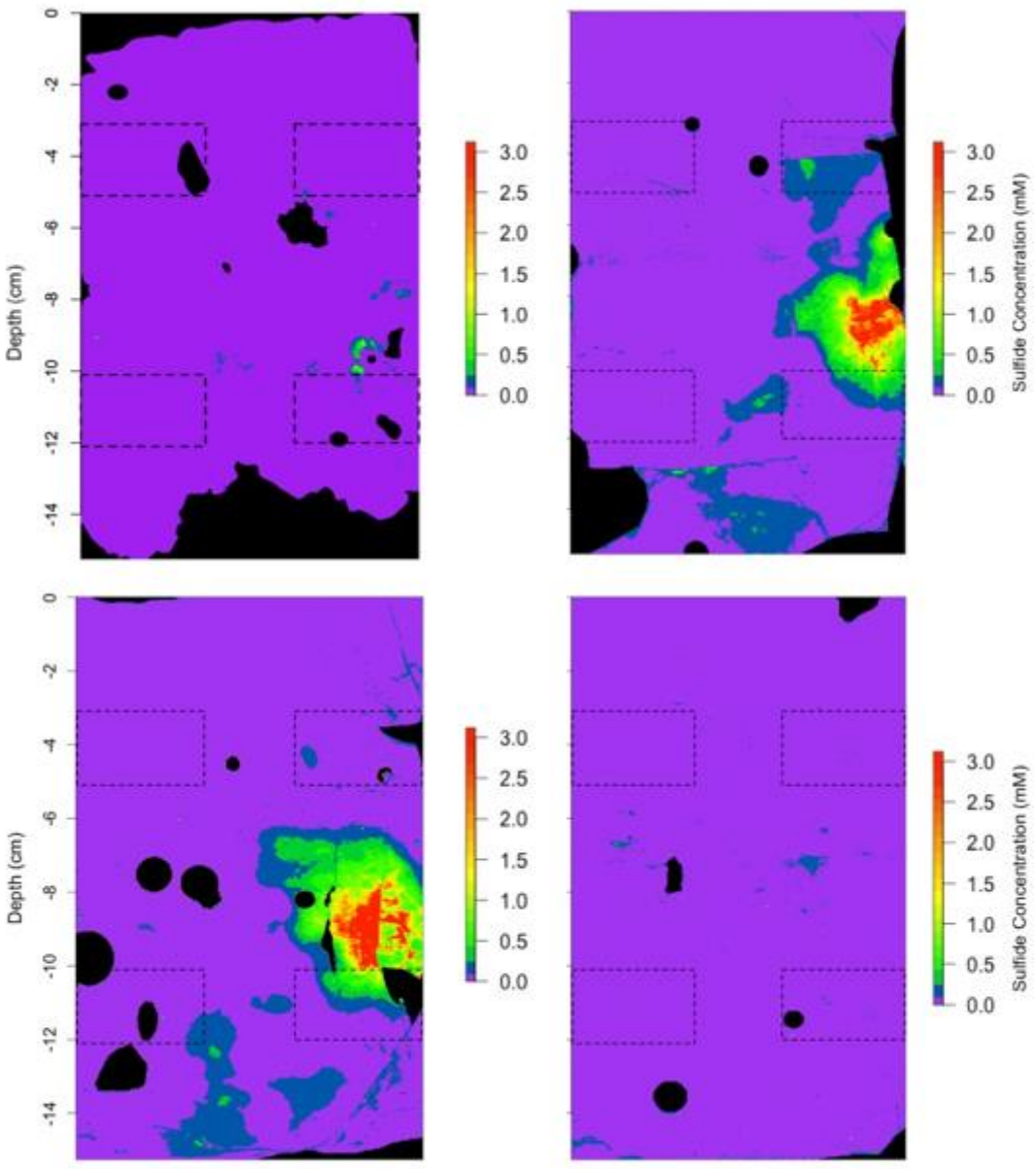

Figure A6. DGT sulfide concentrations for aquarium with no live eelgrass and 0 detritus leaves (S0). The dotted black rectangles show the areas calculated as controls for detritus. Week 1 through week 4 are shown from top left to bottom right.

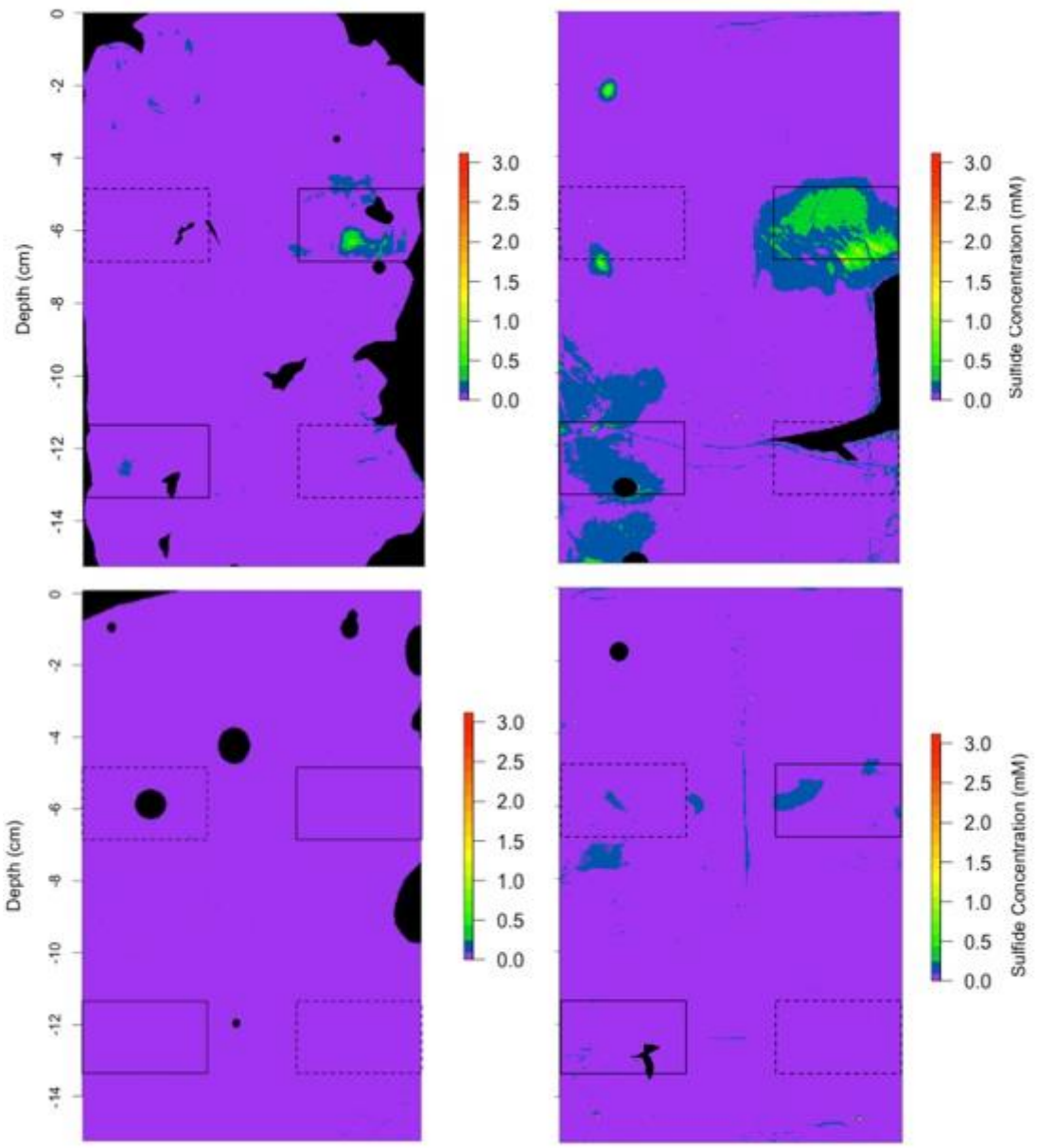

Figure A7. DGT sulfide concentrations for aquarium with no live eelgrass and 1 detritus leaf (S1). The dotted black rectangles show the areas calculated as controls for detritus, whereas the solid black rectangles show the areas where sulfide concentrations from detritus were calculated. Week 1 through week 4 are shown from top left to bottom right.

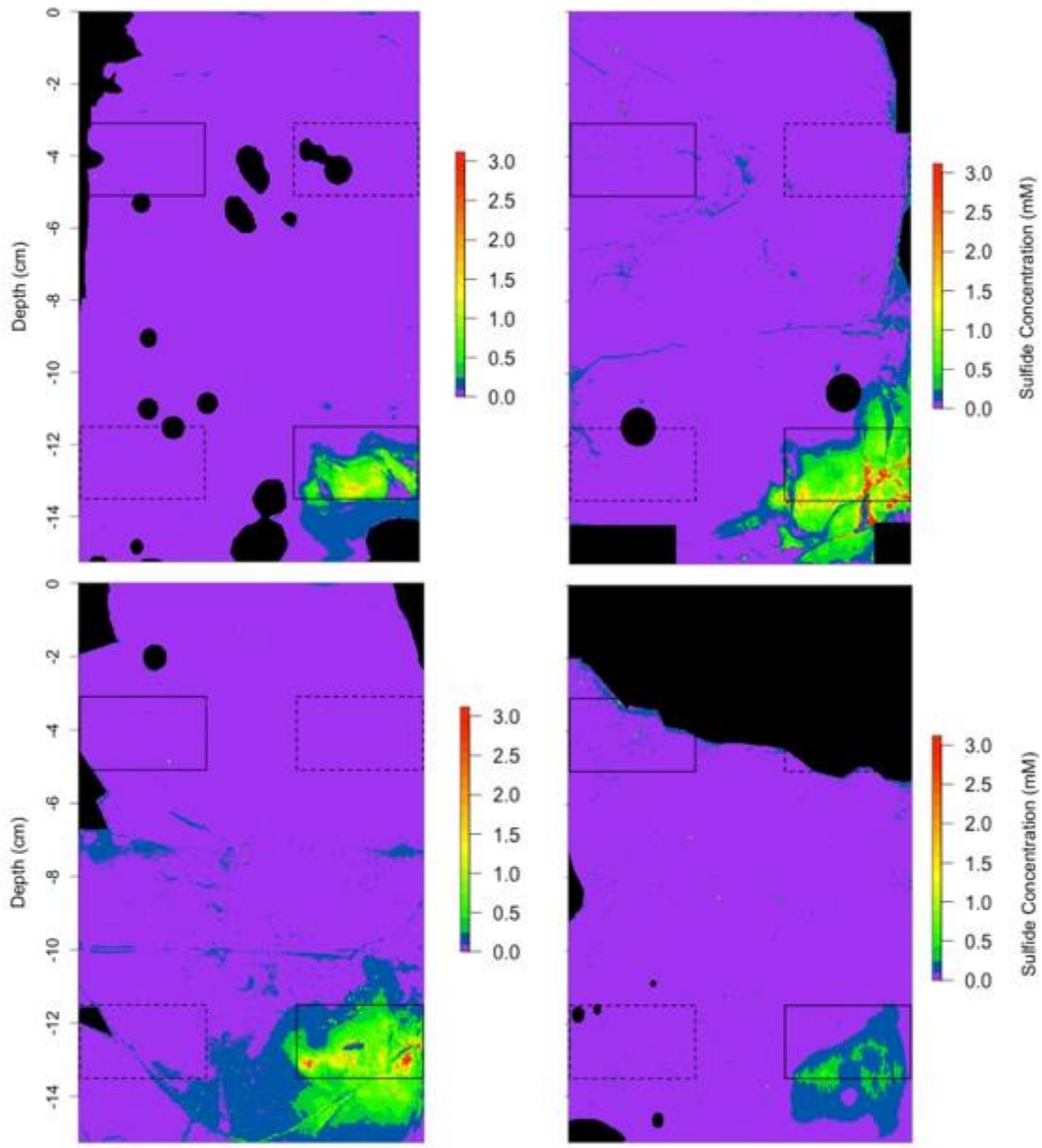

Figure A8. DGT sulfide concentrations for aquarium with no live eelgrass and 2 detritus leaves (S2). The dotted black rectangles show the areas calculated as controls for detritus, whereas the solid black rectangles show the areas where sulfide concentrations from detritus were calculated. Week 1 through week 4 are shown from top left to bottom right.

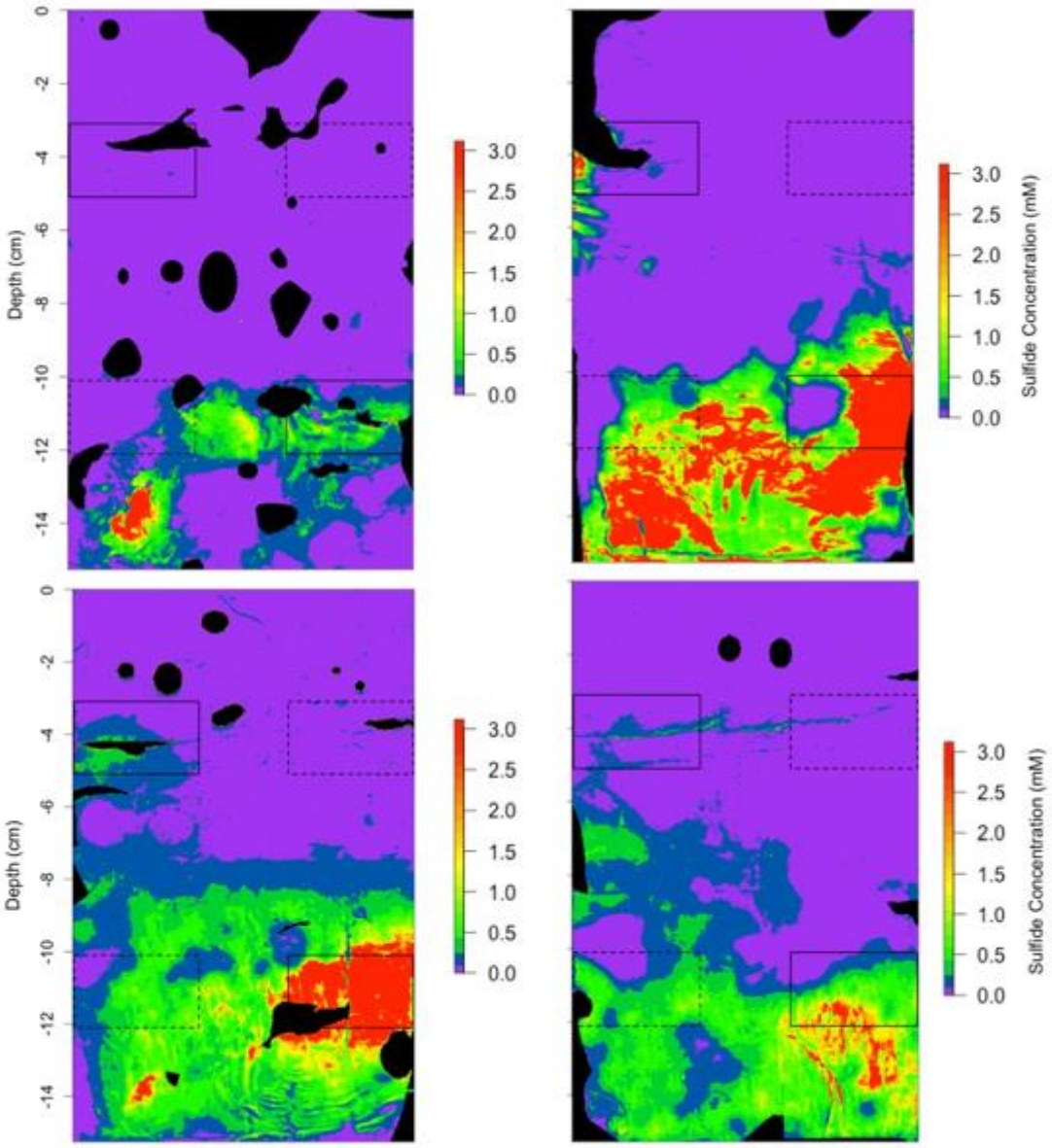

Figure A9. DGT sulfide concentrations for aquarium with no live eelgrass and 4 detritus leaves (S4). The dotted black rectangles show the areas calculated as controls for detritus, whereas the solid black rectangles show the areas where sulfide concentrations from detritus were calculated. Week 1 through week 4 are shown from top left to bottom right.

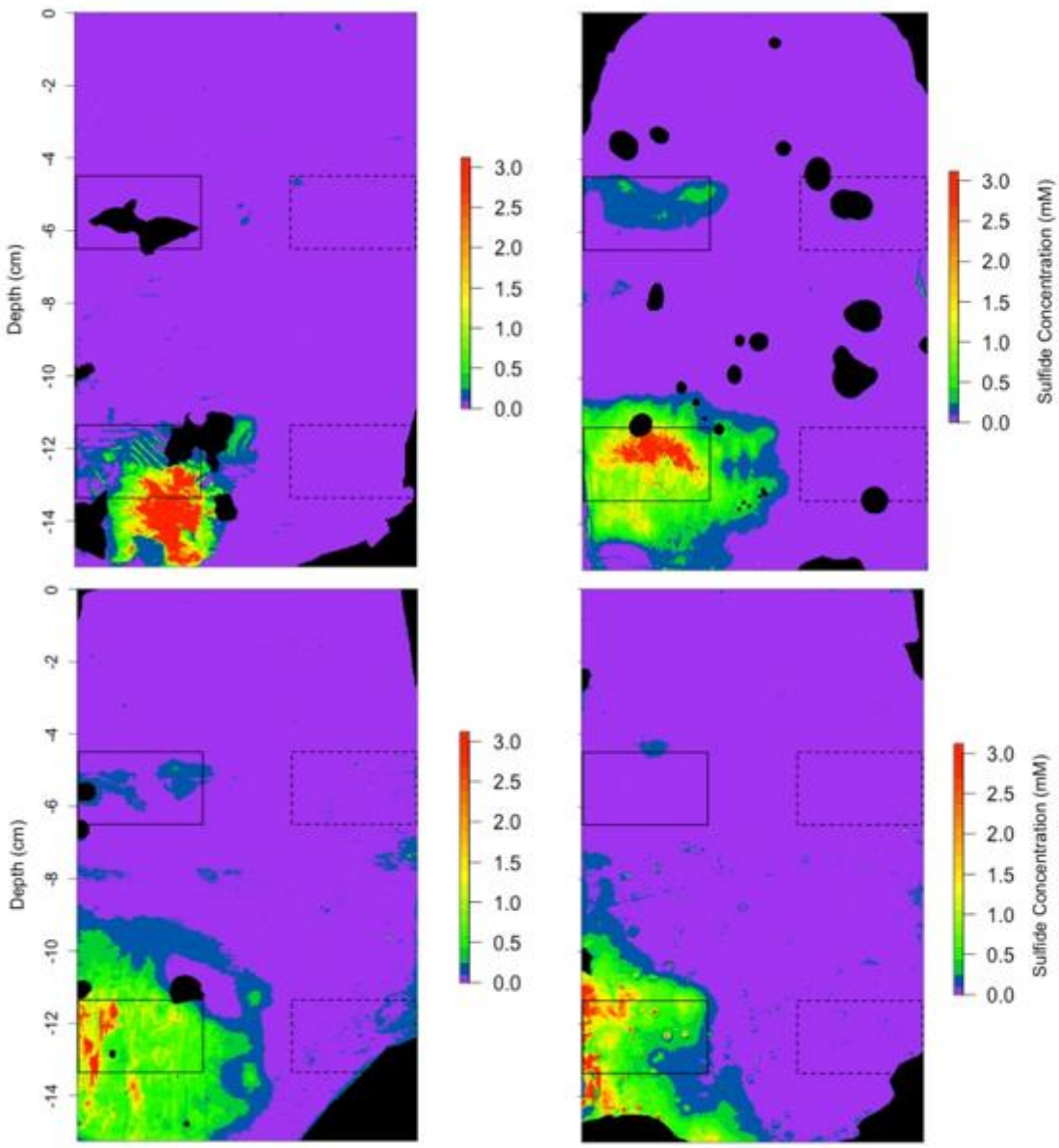

Figure A10. DGT sulfide concentrations for aquarium with no live eelgrass and 8 detritus leaves (S8). The dotted black rectangles show the areas calculated as controls for detritus, whereas the solid black rectangles show the areas where sulfide concentrations from detritus were calculated. Week 1 through week 4 are shown from top left to bottom right.